## REMARKS

Status of the Claims

Claims 36, 39, 42-60, 62-69, and 72-79 are currently pending. Claims 1-35, 37, 38, 40, 41, 61, 70, and 71 have been canceled without prejudice or disclaimer of the subject matter claimed therein. Claims 64-66 are withdrawn from consideration as being directed to a non-elected species. Claims 36, 39, 42-60, 65-69, and 72-79 are under examination.

Claims 36, 44, 52, 55, and 57 have been amended. Representative support for the amendments to claims 36, 44, 52, 55, and 57 can be found in canceled claim 38, and in the specification on page 8, lines 4 and 5, and in Example 2.

Claim 39 has been amended to correct the dependency of the claim.

Claims 59 and 62 have been amended for consistency in the language of the claims.

Claim 63 has been amended to conform with U.S. patent practice, and new claims 78 and 79 have been added. Representative support for new claims 78 and 79 can be found in original claim 63.

The amendments to the claims do not introduce prohibited new matter.

Applicants reserve the right to pursue canceled subject matter in a continuation or divisional application.

Species Election

It is Applicants' understanding that in response to a species election, the Examiner will begin by searching the elected species and will continue searching until art is found or until a generic claim is found allowable. Applicants point out that when a generic claim is found to be allowable, the withdrawn claims which depend from or include the limitations of the allowed claim must be rejoined and fully examined for patentability (see MPEP 809).

The Office Action alleges that MPEP 821.01 requires the cancelation of nonelected claims. Applicants respectfully submit that MPEP 821.01 does not require the cancelation of nonelected claims in response to a final rejection. Moreover, the withdrawn claims, claims 64-66, of the present application, are withdrawn from consideration because they are directed to nonelected species .

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 52-61 and 74-76 are rejected under 35 U.S.C. § 112, second paragraph, as

allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Office Action alleges that claims 52, 55, and 57 are unclear because these claims recite "the active agent" and "paclitaxel." Claims 52, 55, and 57 have been amended to only recite "paclitaxel."

Rejection Under 35 U.S.C. § 103(a)

Claims 36-63 and 67-77 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over McDonald (U.S. Patent 7,112,338) in view of Rahman (U.S. Patent 6,146,659).

Prior to the present invention, the conventional method of treating human patients with paclitaxel is to administer up to the maximum tolerated dose (MTD) of paclitaxel in an attempt to eradicate all tumor cells as quickly and completely as possible. However, the conventional method of administering MTD of paclitaxel causes severe side effects because of the large amount of paclitaxel administered to the patient. Ibrahim reported administering nanoparticle formulation of albumin bound paclitaxel to breast cancer patients at doses of 175 $mg/m^2$ and 300 $mg/m^2$ in a phase II study (see attached, Ibrahim *et al.* 2002, Proc. Am. Soc. Clin. Oncol. 21: Abstr 209).

Moreover, prior to the present invention, in the phase I study of liposome encapsulated paclitaxel (LEP) (the first liposomal taxane that entered clinical trials), the doses of paclitaxel in the LEP formulation ranged from 90 $mg/m^2$ to 300 $mg/m^2$ (see attached, Bowden *et al.*, Proc. Am Soc. Clin. Oncol. 2002, 21:Abstr 1862; Treat *et al.*, Oncology 2001, 15, 44-48)). Further, prior to the present invention, clinical studies for liposomal formulations containing paclitaxel suggest administering such formulations in doses ranging from 90 to 150 $mg/m^2$ (see attached, Soepenberg *et al.*, Eur. J. Cancer, 40 (2004) 681-688). According to the data reported in Soepenberg, myelosuppression was seen at the 150 $mg/m^2$/week dose level. Therefore, Soepenberg recommended dosages of paclitaxel to be in the range of 150 to 180 $mg/m^2$/wk.

Applicants point out that the conversion of $mg/m^2$ to mg/kg (the units recited in the claims of the present application) can be performed using the Food and Drug Administration dose calculator available at http://www.accessdata.fda.gov/scripts/cder/onctools/animalquery.cfm. Table 1 below shows conversion results of some of doses reported in the references cited above.

Table 1: FDA Dose Calculator Results

| Species | Weight (kg) | Dose (mg/kg) | Dose ($mg/m^2$) |
|---|---|---|---|
| Human | 70 | 2.31 | 90 |
| Human | 70 | 4.49 | 175 |
| Human | 70 | 7.70 | 300 |

Moreover, prior to the present invention, there was no reason to use cationic liposomes for delivery of agents into humans because Filion (Filion *et al*, Int. J. Pharma. 162 (1998) 159-170, submitted with Information Disclosure Statement of February 22, 2011)) reported that cationic liposomes are extremely toxic following oral administration and that cationic liposomes induce a large number of adverse effects. Therefore, according to Filion, cationic liposomes are not appropriate for DNA or drug delivery.

The inventors of the present application unexpectedly discovered through clinical studies that administering cationic liposomal preparation comprising low doses of paclitaxel is effective in treating human patients without the severe side effects caused by the conventional methods. The present claims are directed to a novel and unobvious method of treating a human patient comprising administering a cationic liposomal preparation comprising a low dose of paclitaxel.

The Office Action alleges that McDonald teaches the use of liposomally delivered paclitaxel for treating cancer. However, McDonald does not teach or suggest administering cationic liposomal preparations containing low doses of paclitaxel to a human patient. McDonald also does not teach the amount of paclitaxel to administer in a single dose and the amount of paclitaxel to administer in a month. McDonald does not teach or suggest the claimed method of administering paclitaxel.

The Office Action alleges that Rahman teaches dosing ranges and dosing schedules for liposomally encapsulated paclitaxel. However, Rahman does not disclose examples of methods of administering cationic liposomal preparations of low doses of paclitaxel to human patients. Rather, Example 1 of Rahman only discloses preparing liposomal compositions comprising neutral lipids and paclitaxel. Moreover, Rahman teaches administering high doses of taxane. Claim 1 and the Summary of Invention of Rahman recites administering taxane over a period of

less than an hour in an amount of from about 75 to 300 mg/m$^2$ which is equivalent to 1.93 to 7.7 mg/kg body weight for a human of 70 kg bodyweight (see Table 2 below and attached conversion results; conversion performed using the Food and Drug Administration (FDA) dose calculator available at http://www.accessdata.fda.gov/scripts/cder/onctools/animalquery.cfm). Rahman's dosage range is much higher than that recited in the claims.

In column 4, lines 6-10, Rahman alleges that 50 to 300 mg/m$^2$ of an active compound is equivalent to 0.5 to 5 mg/kg body weight of the active compound for a human of 70 kg bodyweight. Applicants respectfully point out that this conversion is incorrect because according to the FDA dose calculator, 50 to 300 mg/m$^2$ is equivalent to 1.28 to 7.7 mg/kg body weight for a human of 70 kg bodyweight (see Table 2 below and attached conversion results). Moreover, Rahman does not disclose examples for administering paclitaxel in the dosage range of 0.5 to 5 mg/kg body weight. Accordingly, the recitation of dosages of an active compound of 0.5 to 5 mg/kg body weight is an inadvertent error.

Moreover, Applicants submit that published PCT application WO 00/01366, which claims priority to the application from which Rahman was granted, only discloses experimental data for administering paclitaxel in the dosage range 90 to 300 mg/m$^2$ to human patients (see Example 2 of attached WO 001366). WO 00/01366 does not disclose experimental data for administering paclitaxel in the dosage range of 0.5 to 5 mg/kg body weight. Further, the claims of U.S. Patent 7,314,637 ('637), which is the national phase application of WO 00/01366, are directed to methods of administering liposomal preparation containing 135 to 300 mg/m$^2$ dosages of taxane (see attached, U.S. Patent 7,314,637), which is equivalent to 3.47 to 7.7 mg/kg body weight for a human of 70 kg body weight (see Table 2 below and attached conversion results) and is much higher than 0.5 to 5.0 mg/kg body weight, disclosed in Rahman. Accordingly, the conversion in column 4, lines 6-10 of Rahman from 50 to 300 mg/m$^2$ to a 0.5 to 5 mg/kg body weight for a human of 70 kg bodyweight is incorrect.

Accordingly, the combination of McDonald and Rahman does not render the claims obvious because neither McDonald nor Rahman teaches or suggests administering cationic liposomal preparation comprising low dosages of paclitaxel and neither McDonald nor Rahman teaches or suggests the dosages recited in the claims. Moreover, McDonald and Rahman use different dosages of paclitaxel in their liposomal formulations because McDonald and Rahman teach using different types of liposomes for delivering taxanes to a patient. Therefore, there is no reason to combine the teachings of McDonald and Rahman.

Further, the presently claimed methods of treating human patients are based on clinical studies. As is well known, clinical studies provide data from a human patient for determining efficacy and safety or toxicity of dosages of compounds, which require extensive time and money to conduct. Neither McDonald nor Rahman discloses clinical data for the efficacy and safety of the dosages of paclitaxel. Also, the cited references provide no reason to use a low dose of paclitaxel in treating human patients, as recited in the present claims. Accordingly, McDonald and Rahman provide no reason to modify the teachings of the prior art of administering MTD of paclitaxel to a patient to arrive at the claimed invention with a reasonable expectation of success.

Applicants respectfully submit that *In re Aller* is not applicable to the present case because *In re Aller* is directed to optimizing parameters by routine experimentation based on known information. In contrast, the inventors of the present application unexpectedly discovered through clinical trials, that a low dose of paclitaxel can be effectively administered to a human patient using cationic liposomes. As mentioned, prior to the present invention, there was no known safe and effective administration schedule for paclitaxel because the thought was to use high doses of paclitaxel to treat patients. However, these high doses of paclitaxel induced severe side effects. Therefore, the doses of paclitaxel used in clinics, prior to the present invention, were not safe and/or effective.

Unlike the situation in *Aller*, the clinical trials conducted by the present inventors were not routine experimentation for optimizing dosages of administering paclitaxel, since there were no known safe and effective dosage for administering paclitaxel to optimize. The inventors unexpectedly discovered through clinical trials that cationic liposomal formulations containing a low dose of paclitaxel are effective in treating human patients. Applicants also point out that cationic liposomal formulations containing paclitaxel were not commercially available at the time of the invention. Moreover, prior to the present invention, it was thought that cationic liposomes were toxic to patients. Accordingly, it was not routine experimentation to conduct the clinical trials for finding an effective dosage of paclitaxel for treating patients.

Table 2: FDA Dose Calculator Results

| Species | Weight (kg) | Dose (mg/kg) | Dose ($mg/m^2$) |
|---|---|---|---|
| Human | 70 | 1.28 | 50.00 |

| | | | |
|---|---|---|---|
| Human | 70 | 1.93 | 75.00 |
| Human | 70 | 3.47 | 135 |
| Human | 70 | 7.70 | 300 |

Obviousness-Type Double Patenting

A. Claims 36-63 and 67-77 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 9, 11, 14, 18, and 20 of U.S. Patent 7,794,747 ('747).

Applicants respectfully submit that the claims of the present application and the claims of '747 are directed to patentably distinct inventions. The claims of the present application are directed to novel and unobvious methods of treating a human patient comprising administering a cationic liposomal composition comprising paclitaxel at a specific dosage, while the claims in '747 are directed to novel and unobvious methods of making a cationic liposomal composition comprising a taxane.

The Office Action alleges that claim 16 of the patent is directed to liposomes. Applicants respectfully point out that claim 16 is dependent on claim 1 which is directed to a method of making a cationic liposomal preparation comprising a taxane. Accordingly, claim 16 is directed to a method of making a cationic liposomal preparation comprising the features recited in claim 16.

The Office Action alleges that '747 discloses the use of the liposomes to treat cancer. However, '747 does not claim the use of the cationic liposomal preparation for treating cancer, and '747 does not teach or suggest the specific dosages recited in the claims of the present application.

The Office Action alleges that based on the decision in *In re Ochiai,* a given compound renders its methods of making and using obvious. Applicants respectfully point out that *In re Ochiai* is not relevant to the present obviousness-type double patenting rejection. In *Ochiai,* the Court reviewed the issue of whether a known method of making a novel and nonobvious product is patentable and concluded that a known method of making a novel and nonobvious product is patentable. Accordingly, the Court in *Ochiai* did not hold that a given compound renders its methods of making and using obvious.

As discussed above, the claims in '747 and the claims in the present applications are directed to patentably distinct novel and nonobvious methods of making and using cationic liposomal compositions comprising taxane and paclitaxel, respectively. The claims of '747 are directed to an improved method for making a cationic liposomal composition comprising taxane, while the claims of the present application are directed to a novel and unobvious method of administering cationic liposomal compositions comprising specific dosages of paclitaxel for effective treatment of human patients without severe side effects.

B. Claims 36-63 and 67-77 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 13, 16, and 22 of copending U.S. Patent Application 12/300,448.

U.S. Patent Application 12/300,448 is no longer pending. Accordingly, this provisional rejection is deemed moot.

C. Claims 36-63 and 67-77 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 4, 5, and 9 of copending U.S. Patent Application 12/308,748.

U.S. Patent Application 12/308,748 is no longer pending. Accordingly, this provisional rejection is deemed moot.

Technically Related Cases

Applicants provide the status of pending U.S. applications with the same assignee that may be considered to be technically related for the Examiner's consideration.

| Application No. | Filing Date | Status |
|---|---|---|
| 11/919,700 | Oct. 31, 2007 | Pending |
| 12/293,039 | Jan. 9, 2009 | Pending |

Conclusion

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request entry of the amendments, reconsideration, and the timely allowance of the pending claims. A favorable action is awaited. Should an interview be helpful to further prosecution of this application, the Examiner is invited to telephone the undersigned.

If there are any additional fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 13-3250. If a fee is required for an extension of time under 37 C.F.R. §1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
**MILBANK, TWEED, HADLEY & McCLOY LLP**

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# Nanoparticle paclitaxel (ABI-007) in metastatic breast cancer (MBC): efficacy and evidence of dose-dependent activity in two multicenter phase II studies

**Sub-category:**
Breast Cancer - Metastatic Breast Cancer

**Category:**
Breast Cancer

**Meeting:**
2002 ASCO Annual Meeting

**Session Type and Session Title:**
General Poster Session, Breast Cancer

**Abstract No:**
209

**Citation:**
Proc Am Soc Clin Oncol 21: 2002 (abstr 209)

**Author(s):**
Nuhad K Ibrahim, Brian Samuels, Ray Page, Troy Guthrie, G. Ramakrishnan, D. Doval, K. Patel, S. Rao, M. Nair, D. Digumarti, Gabriel N Hortobagyi, UT MD Anderson Cancer Ctr, Houston, TX; Lutheran General Cancer Ctr, Park Ridge, IL; Texas Cancer Ctr Care, Fort Worth, TX; University of Florida, Jacksonville, FL; Tata Memorial Hospital, Bombay, India; Rajiv Gandhi Cancer Institute, New Delhi, India; Gujarat Cancer Research Institute, Ahmedabad, India; MNJ Institute of Oncology, Hyderabad, India; Regional Cancer Center, Thiruvananthapuram, India; Nizams Institute of Medical Sciences, Hyderabad, India.

**Abstract:**

ABI-007 is a novel albumin-stabilized, cremophor-free, nanoparticle formulation of paclitaxel. In a phase I study (Ibrahim et al, ASCO 2000), the MTD of ABI-007 was determined to be 300 mg/m2 by 30 minute infusion every 3 weeks, without premedication or G-CSF support. Therefore, 2 multicenter phase II studies were conducted to evaluate the efficacy of 2 dose levels of this paclitaxel formulation: 300mg/m2, study(S)1 and 175 mg/m2, S2. The inclusion criteria and design of both studies were similar except for the dose. Between Nov 1999 and May 2001, for S1 and S2 respectively, patients (pts) enrolled: 63 and 43; number of treatment cycles administered per pt, median (M)(Range(R)): 4.5(1-13) and 6(1-10); number of disease sites M(R): 3(1-9) and 2(1-7); Prior anthracycline (A) and prior taxane (T) therapy was noted. [table] Hematologic toxicities in S1 and S2, respectively: absolute neutrophil count (ANC) <2000/mm3: 65% and 16%; ANC <500/mm3: 23% and 5%; thrombocytopenia (TC) <100,000/mm3: 7% and 2%; TC <50,000/mm3: 5% and 2%; febrile neutropenia: 3 pts, 2 hospitalized; 1 pt, hospitalized. NCI Grade 3/4 non-hematologic toxicities in S1 and S2, respectively: peripheral neuropathy: 10% and 0%; Myalgia: 5% and 0%; Vomiting: 2% and 2%; Diarrhea: 3% and 0%; Rash: 2% and 0%; Amblyopia: 2% and 0%. No allergic reactions. We conclude that ABI-007 is an efficacious and well tolerated agent for MBC. A pivotal phase III trial to confirm this data is ongoing.

## Results:

| Responses N(%): | | | | | |
|---|---|---|---|---|---|
| Dose | ORR* | CR | PR | SD | PD |
| 300 mg/m2 (N=59) | 36 (61) | 2(3) | 34 (58) | 11 (19) | 12 (20) |
| A Naive (N=29) | 22 (76) | 1(3) | 21 (72) | 5(17) | 2(7) |
| Prior A (N=29) | 14 (48) | 1(3) | 13 (45) | 6(21) | 9 (31) |
| Prior T (N=7) | 3(43) | 0(0) | 3(43) | 2(29) | 2 (29) |
| | | | | | |
| 175 mg/m2 | 21 | 3(7) | 18 | 15 | 5 |

| | | | | | |
|---|---|---|---|---|---|
| (N=41) | (51) | | (44) | (37) | (12) |
| A Naive (N=18) | 9(50) | 0(0) | 9(50) | 8(44) | 1(6) |
| Prior A (N=22) | 11 (50) | 3(14) | 8(36) | 7(32) | 4 (18) |
| Prior T (N=2) | 0(0) | 0(0) | 0(0) | 2 (100) | 0(0) |

*ORR=overall response rate, median time to progression not reached.

▸ **Associated Presentation(s):**

1. Nanoparticle paclitaxel (ABI-007) in metastatic breast cancer (MBC): efficacy and evidence of dose-dependent activity in two multicenter phase II studies

Meeting: 2002 ASCO Annual Meeting
Presenter: Nuhad K. Ibrahim
Session: Breast Cancer (General Poster Session)

▸ **Other Abstracts in this Sub-Category:**

1. Preserved chemosensitivity to weekly paclitaxel and carboplatin in HER2+ patients irrespective of responses to first-line intensified induction herceptin single agent therapy

Meeting: 2002 ASCO Annual Meeting Abstract No: 127 First Author: Denise A Yardley
Category: Breast Cancer - Breast Cancer - Metastatic Breast Cancer

2. Inhibition of epidermal growth factor/HER2 receptor signaling using ZD1839 ("Iressa") restores tamoxifen sensitivity and delays resistance to estrogen deprivation in HER2-overexpressing breast tumors

Meeting: 2002 ASCO Annual Meeting Abstract No: 130 First Author: S Massarweh
Category: Breast Cancer - Breast Cancer - Metastatic Breast Cancer

3. Letrozole (Femara) vs. anastrozole (Arimidex): second-line treatment in postmenopausal women with advanced breast cancer

Meeting: 2002 ASCO Annual Meeting Abstract No: 131 First Author: C Rose
Category: Breast Cancer - Breast Cancer - Metastatic Breast Cancer

More...

▸ **Abstracts by Nuhad K Ibrahim:**

1. Open label, randomized clinical trial of standard neoadjuvant chemotherapy with paclitaxel followed by FEC (T-FEC) versus the combination of paclitaxel and RAD001 followed by FEC (TR-FEC) in women with triple receptor-negative breast cancer (TNBC).

Meeting: 2011 ASCO Annual Meeting Abstract No: 1016 First Author: A. M. Gonzalez-Angulo

Category: Breast Cancer - Triple-negative/Cytotoxics/Local Therapy - Triple Negative Breast Cancer

2. Seventy-two month update of randomized trial of preoperative therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy with or without concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer

Meeting: 2009 Breast Cancer Symposium Abstract No: 221 First Author: A. Buzdar
Category: Treatment - Preoperative Study Methods

3. Phase I study of bavituximab, a novel anti-phosphatidylserine monoclonal antibody in patients with advanced refractory cancer: Preliminary results.

Meeting: 2009 ASCO Annual Meeting  Abstract No: 1080  First Author: N. K. Ibrahim
Category: Breast Cancer - Metastatic Breast Cancer - Metastatic Breast Cancer

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▸ **Presentations by Nuhad K Ibrahim:**

1. Phase I study of bavituximab, a novel anti-phosphatidylserine monoclonal antibody in patients with advanced refractory cancer: Preliminary results.

Meeting: 2009 ASCO Annual Meeting
Presenter: Nuhad K Ibrahim, MD
Session: Breast Cancer - Metastatic (General Poster Session)

2. A nomogram to predict subsequent brain metastasis in metastatic breast cancer (MBC) patients.

Meeting: 2008 ASCO Annual Meeting
Presenter: Nuhad K Ibrahim, MD
Session: Breast Cancer — Metastatic (Poster Discussion Session)

3. Clinicopathological correlation (CC) and outcome of breast cancer patients (pts) with resected brain metastasis (BM).

Meeting: 2006 ASCO Annual Meeting
Presenter: Nuhad K Ibrahim
Session: Breast Cancer (General Poster Session)

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▸ ***Educational Book Manuscripts by Nuhad K Ibrahim*:**

No items found.

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# Phase 1 trial in advanced malignancies with liposome encapsulated paclitaxel (LEP) Q 3 weeks.

**Sub-category:**
Biologic and Targeted Therapies

**Category:**
Biologic and Targeted Therapies

**Meeting:**
2002 ASCO Annual Meeting

**Session Type and Session Title:**
This abstract will not be presented at the 2002 ASCO Annual Meeting but has been published in conjunction with the meeting.

**Abstract No:**
1862

**Citation:**
Proc Am Soc Clin Oncol 21: 2002 (abstr 1862)

**Author(s):**
Chris Bowden, Chao Huang, P. Eisenberg, Douglas Reding, A. Giancarli, C. Giorgetti, P. Angiuli, Pharmacia, Nerviano, Italy; Fox Chase-Temple Cancer Center, Philadelphia, PA; Marin Oncology Associates, Marin, CA; Marshfield Clinic, Marshfield, WI.

**Abstract:**

LEP is the first liposomal taxane to enter clinical trials. The first component of this trial used a formulation requiring sonication prior to administration (ASCO, 2000, #881). A new formulation has been developed and is undergoing further testing in standard Phase I dose escalation. To date, 16 patients (pts) have been treated with the new formulation, Q 21 days at dose levels of 200 mg/m2 (n=4), 225 mg/m2 (n=3), 250 mg/m2 (n=3), 275 mg/m2 (n=3) and 300 mg/m2 (n=3). All patients have received prior chemotherapy or immunotherapy. Hematologic toxicities: febrile neutropenia has been reported by 1 pt at 200 mg/m2 (C2), 1 pt at 275 mg/m2 (C4), and 1 pt at 300 mg/m2 (C1; DLT). Dose limiting non-hematologic toxicity has not been reported. Grade 2 neuropathy/paresthesia has been reported by 1 patient at 275 mg/m2 and 300 mg/m2. Mild to moderate liposome infusion reactions have occurred in 5 out of 16 pts. A slow rate of infusion for the first 15 minutes has enabled the majority of patients to complete infusion in approximately 1 hr. Free (non liposome associated, bound and unbound to plasma protein) and total (free plus liposome associated) paclitaxel were assayed in plasma and blood, respectively with two validated HPLC/MS/MS methods(LLOQ: 1 and 5 ng/ml for free and total, respectively). Free paclitaxel showed a polyexponential decline with a terminal half-life of 185-196 h, longer than that reported for paclitaxel after Taxol administration. Overall, in terms of AUC, free paclitaxel levels were representing about 6-24% of the total. LEP is well tolerated at doses up to 300 mg/m2, in contrast to the prior formulation which had an MTD of 175 mg/m2. Preliminary pharmacokinetics data indicates LEP provides continuous exposure to free paclitaxel over a prolonged period. Enrollment and pharmacokinetics analysis is ongoing.

▸ **Associated Presentation(s):**

No items found.

▸ **Other Abstracts in this Sub-Category:**

1. KIT mutational status predicts clinical response to STI571 in patients with metastatic gastrointestinal stromal tumors (GISTs)

Meeting: 2002 ASCO Annual Meeting Abstract No: 6 First Author: Michael C Heinrich
Category: Biologic and Targeted Therapies

2. A rationally designed, targeted tumor treatment approach: a phase II study of imatinib mesylate (Gleevec) in patients with life threatening diseases known to be associated with imatinib-sensitive tyrosine kinases

Meeting: 2002 ASCO Annual Meeting Abstract No: 7 First Author: Jane Apperley
Category: Biologic and Targeted Therapies

3. Hairy cell leukemia disease burden and complete remission with BL22, a recombinant immunotoxin targeting CD22

Meeting: 2002 ASCO Annual Meeting Abstract No: 8 First Author: Robert J Kreitman
Category: Biologic and Targeted Therapies

More...

▸ **Abstracts by Chris Bowden:**

1. Impact of dalteparin low-molecular-weight heparin (LMWH) on survival: Results of a randomized trial in cancer patients with venous thromboembolism (VTE)

Meeting: 2003 ASCO Annual Meeting Abstract No: 846 First Author: A. Y. Lee
Category: Developmental Therapeutics - Experimental Therapeutics - Antiangiogenic Agents

2. A PHASE I AND PHARMACOKINETIC (PK) STUDY OF THE FARNESYLTRANSFERASE INHIBITOR, R115777 IN COMBINATION WITH GEMCITABINE (Gem).

Meeting: 2000 ASCO Annual Meeting Abstract No: 5A First Author: Amita Patnaik
Category: Developmental Therapeutics - Developmental Therapeutics - Clinical Pharmacology and Immunotherapy

3. A study of anti-epidermal growth factor receptor (EGFr) monoclonal antibody C225 and cisplatin in patients (pts) with head and neck or lung carcinomas (Meeting abstract).

Meeting: 1997 ASCO Annual Meeting Abstract No: 1364 First Author: J Falcey
Category: Head and Neck and CNS

More...

▸ **Presentations by Chris Bowden:**

No items found.

▸ ***Educational Book Manuscripts by Chris Bowden*:**

No items found.

# Liposomal-Encapsulated Chemotherapy: Preliminary Results of a Phase I Study of a Novel Liposomal Paclitaxel

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**ABSTRACT**

***Liposome encapsulation of antineoplastic drugs entered clinical testing in the late 1980s. As carriers for a variety of agents, liposomes can allow successful delivery of agents that may be subject to rapid degradation in the serum and can modify the toxicity profile. In general, liposomes have demonstrated an ability to attenuate toxicities by their different pharmacokinetic profile and pattern of distribution. Differences in the constitution of the liposome can greatly affect the pharmacokinetic profile resulting in different patterns of toxicity. Characteristics such as size, charge, composition, and integrity can affect performance of the liposome. Liposome encapsulation of doxorubicin has been shown to reduce cardiac toxicity. Preliminary data suggest that encapsulation of paclitaxel can greatly modify neurotoxicity without the need for cremephor.***

The majority of anticancer drugs are nonspecific, cytotoxic agents that damage tumor cells as well as normal healthy tissue. Given their narrow therapeutic index, antineoplastic drugs have the potential for serious side effects. A variety of drug-delivery systems are currently being used in an effort to make anticancer agents more efficient and less toxic. Liposomal encapsulation of chemotherapeutic agents represents one method of achieving this goal.

## Ideal Candidates for Drug-Delivery Systems

First described by Bangham more than 30 years ago,[1] liposomes have undergone significant refinements over the decades to evolve into important modern-day drug carriers. Liposomes are spherical vesicles comprised of an outer phospholipid membrane with an internal aqueous compartment. Water-soluble drugs can be contained in the aqueous compartment while hydrophobic drugs incorporate within the lipid bilayer.

Liposomes are ideal candidates for drug-delivery systems. As a result of their similarity to biological membranes, they are safe, biocompatible, and biodegradable. Liposomes are extremely versatile macromolecules. By manipulating certain physical parameters such as phospholipid composition, size, or membrane characteristics, liposomes can be engineered to efficiently encapsulate and effectively transport a variety of drugs. Small changes in these parameters can also have profound effects on the pharmacokinetic and pharmacodynamic profiles of a drug.[2-9]

The advancement of the liposomal drug-delivery system in the oncology field was predicated on preclinical and clinical data demonstrating the benefits of liposomal encapsulation of chemotherapeutic agents. Some of the liposomal preparations significantly increase the half-life of the incorporated agent vs that of the free drug.[10-13]

Shielding the free drug within a liposome protects it from plasma protein interaction and metabolic degradation. As a result, pharmacokinetic parameters of the free drug are altered by liposomal encapsulation. Indeed, chemotherapeutic agents incorporated into liposomes exhibit longer circulation lifetimes with a greater area under the curve, lower rate of clearance, and smaller volume of distribution as compared with that of the free drugs.[13–19] The narrow therapeutic

index of many small-molecule chemotherapeutic drugs is, in part, a consequence of their large volume of distribution. Incorporation into liposomes significantly reduces the volume of distribution, thereby decreasing the toxicity to normal tissue and increasing the amount of drug that can be effectively delivered to the tumor.[20,21]

## *Advantages of Liposomal Encapsulation of Doxorubicin*

The anthracycline doxorubicin is one of the most important cytotoxic agents used in anticancer therapy. It has significant clinical activity in hematologic malignancies, such as lymphomas and leukemias, as well as in numerous solid tumors. However, our ability to achieve maximum clinical efficacy with doxorubicin is limited by the development of irreversible cardiotoxicity and multidrug resistance (MDR).

Myelosuppression is the dose-limiting toxicity, but cardiotoxicity remains the therapy-limiting toxicity. In order to avoid this complication, therapy is generally discontinued when the cumulative dose reaches 450–500 mg/m$^2$, at which point the risk of cardiotoxicity is significant.[22-24] Liposomes have been successfully employed to circumvent some of the limitations of free doxorubicin. Liposomal encapsulation of doxorubicin significantly alters its pharmacokinetic profile, attenuates its toxicity patterns in clinical settings, and demonstrates in preclinical trials an ability to reverse multidrug resistance.[25-28]

### Alters Pharmacokinetic Profile

Numerous phase I studies have clearly demonstrated significantly higher areas under the curve for liposomal-encapsulated doxorubicin (LED) than for similar intravenous bolus doses of free doxorubicin.[29-32] Free doxorubicin reaches a peak concentration within minutes after administration and has a terminal elimination half-life of 30 hours. Depending on the specific liposomal formulation, doxorubicin-containing liposomes can increase the area under the curve by 20 to 600 times.[31,32] Therefore, the pharmacokinetic pattern of liposomal-encapsulated doxorubicin more closely mimics the prolonged infusion regimen of doxorubicin, which has demonstrated less systemic toxicity, most notably diminished cardiotoxicity and gastrointestinal toxicity.[33]

### Attenuates Toxicity Patterns

Additionally, liposomal-encapsulated doxorubicin exhibits a much smaller volume of distribution as compared with free doxorubicin. The estimated volume of distribution for free doxorubicin is approximately 25 L/kg, or 1,875 liters for a 70-kg person, reflecting significant tissue uptake.[34,35] In contrast, liposomal preparations can reduce the volume of distribution by 10- to 60-fold.[10-12,31,32] The relatively large size of liposomes probably accounts for the marked reduction in the volume of distribution.

These large macromolecules are usually unable to traverse the small endothelial gaps of normal tissue, especially cardiac endothelial cells.[21,36] Indeed, a large body of clinical data supports findings that cancer patients treated with liposomal-encapsulated doxorubicin have a significant reduction in cardiac toxicity.[37-41] Diminished hematologic toxicity (myelosuppression), gastrointestinal toxicity (nausea/vomiting), mucositis, and venous sclerosis have also been noted with liposomal-encapsulated doxorubicin.[37]

### Shown in Preclinical Trials to Reverse Multidrug Resistance

Multidrug resistance is an important impediment that is difficult to circumvent in the treatment of cancer. Overexpression of P-glycoprotein (Pgp170) in neoplastic cells can lead to marked resistance to doxorubicin. Preclinical studies have demonstrated liposomal modulation of MDR.[25-28] In one study, liposomal-encapsulated doxorubicin was shown to prevent effective multidrug resistance by binding to the P-glycoprotein plasma membrane pump expressed by the multidrug resistant genes.[27] This may help explain why liposomal-encapsulated doxorubicin has been effective in the treatment of various chemotherapy-refractory cancers.[42-44]

### Extensively Studied in Treatment of Advanced Breast Cancer

Liposomal doxorubicin is steadily proving to be an important antitumor agent. It has been extensively studied in the treatment of advanced breast cancer, ovarian cancer, and Kaposi's sarcoma.

The first phase II study of liposomal-encapsulated doxorubicin in advanced breast cancer included 20 patients who had not received anthracycline treatment within the past year.[42] Sixteen of the 20 patients received doxorubicin doses of 75 mg/m$^2$ every 3 weeks, while the four remaining patients, all of whom had undergone extensive radiotherapy, received either 45 or 60 mg/m$^2$.

Overall, five patients demonstrated a complete response in their index lesion, while four others demonstrated objective disease regression. The mean duration of response was 7 months.

Most patients had some degree of myelosuppression, but the mean nadir leukocyte count for all the cycles was only 3,740/μL, and no patient developed sepsis or infection. Other toxic effects included two episodes of grade 4 nausea/vomiting, two episodes of mild stomatitis, and complete alopecia in all patients.

Cardiotoxicity was also assessed. Two patients had left ventricular ejection fraction decreases (13% and 17%), and endomyocardial biopsies performed on five patients who received cumulative doses of more than 500 mg/m$^2$ revealed four grade 0 and one episode of grade 1 structural changes. These data demonstrate the clinical activity of liposomal-encapsulated doxorubicin against breast cancer, and suggest that liposomal-encapsulated doxorubicin causes less cardiotoxicity, myelosuppression, and gastrointestinal toxicity than equivalent doses of free doxorubicin.

Subsequent trials in advanced breast cancer have demonstrated good response rates and a favorable toxicity profile, especially reduced cardiotoxicity.[39,45] A recent phase III trial was reported comparing cyclophosphamide (Cytoxan, Neosar) and either TLC D-99 or standard doxorubicin as first-line treatment of metastatic breast cancer.[46] Response rates were 43% in both arms with a median survival of 21.2 months for the TLC D-99 patients and 16.4 months for those receiving standard doxorubicin. As expected, those treated with TLC D-99 had significantly less cardiotoxicity and grade 4 myelosuppression.

### Clinical Response Against Refractory Ovarian Cancer

Favorable results have also been demonstrated in patients with refractory ovarian cancer. A phase II trial of liposomal doxorubicin was conducted in 35 women with ovarian cancer refractory to platinum- and paclitaxel (Taxol)-based regimens.[47] Patients received 50 mg/m² IV every 3 weeks. Nine clinical responses were observed (one complete response and eight partial responses) in 35 patients (25.7% response rate). The median progression-free survival was 5.7 months with an overall survival of 1.5 to 24 + months (median: 11 months).

Four patients developed fever and grade 3 neutropenia, while ten developed grade 3 hand-foot syndrome. No cases of alopecia, cardiotoxicity, phlebitis, or hepatic dysfunction were observed. This result suggests that liposomal doxorubicin might be useful as second-line therapy for this disease, and the lack of moderate-to-severe myelosuppression indicates that it can potentially be integrated into combined drug modalities for ovarian cancer without requiring dose attenuation.

### Favorable Response Among Kaposi's Sarcoma Patients

Patients with Kaposi's sarcoma also respond favorably to this formulation. A number of phase II and III trials have shown liposomal doxorubicin to be an effective agent in the treatment of AIDS-associated Kaposi's sarcoma. Single-agent therapy produced a significantly better overall response rate with much less toxicity and greater patient compliance vs a multidrug regimen.[44,48-50] Future trials, testing the formulation in combination with other active agents, are planned.

## *Liposomal Formulation Attenuates Toxicity of Paclitaxel*

Paclitaxel is an active antineoplastic agent that was derived from the bark extract of the Pacific yew (*Taxus brevifolia*). It promotes assembly and enhances stability of microtubules, the components of the intracellular skeleton, which modulate mitosis as well as other biological functions such as protein secretion, intracellular transport, and cell motility.[51,52] By altering the dynamic nature of microtubule assembly and disassembly, which is critical for entry into and execution of the mitotic cell cycle, paclitaxel leads to functional mitotic arrest of affected cells in the G2 and M phases.[53,54] Numerous clinical studies have demonstrated the activity of paclitaxel in a variety of malignancies, including breast, ovarian, lung, and head and neck tumors.[55-59]

Despite its broad and promising antitumor profile, paclitaxel is associated with many clinically relevant toxic side effects. Peripheral neuropathy is a significant clinical toxicity. Hypersensitivity reactions characterized by dyspnea, hypotension, angioedema, and generalized urticaria as well as cardiac arrhythmias may occur. Other problematic side effects include myalgia and arthralgia, alopecia, nausea, vomiting, diarrhea, mucositis, and phlebitis.[60-62]

Given the potent and extensive antitumor effects of paclitaxel, attenuation of its systemic toxicity would be a significant accomplishment in the field of oncology. Because liposomes have successfully reduced the toxicity profile of various other chemotherapeutic agents while maintaining and sometimes improving efficacy, the application of the liposomal vector to paclitaxel is intriguing.

### Modulates Multidrug Resistance

A liposome-encapsulated paclitaxel preparation has been developed that overcomes the stability and solubility problems associated with conventional paclitaxel. Preclinical evaluations demonstrated that liposome-encapsulated paclitaxel modulates multidrug resistance in human promyelocytic leukemia HL-60/VCR cells by ninefold, and in human ovarian SKVLB cancer cells by eightfold compared with conventional paclitaxel.

Intracellular paclitaxel accumulation in these cell lines was enhanced by threefold to fourfold when presented in liposomes. Liposome-encapsulated paclitaxel also showed a significant reduction in the efflux rate of paclitaxel from the cells as compared with that of conventional paclitaxel.[63]

### Significantly Less Toxic

Another preclinical study was designed to evaluate the pharmacokinetics, tissue distribution, toxicity, and therapeutic efficacy of liposome-encapsulated paclitaxel in comparison to conventional paclitaxel. In normal mice, liposome-encapsulated paclitaxel was much less toxic than standard paclitaxel with comparable antitumor activity. The area under the curve was twofold higher and the elimination half-life was two times longer with liposome-encapsulated paclitaxel than with conventional paclitaxel.

As expected, conventional paclitaxel displayed nonlinear pharmacokinetics with a disproportionate increase in area under the curve compared to the dose administered. Interestingly, at the dose levels studied, liposome-encapsulated paclitaxel demonstrated linear kinetics. Tissue distribution of paclitaxel after administration of liposome-encapsulated paclitaxel showed levels 10-fold higher in the spleen and 3.5-fold higher in the liver as compared to levels achieved with conventional paclitaxel. However, in kidneys, lungs, the brain, and lymph nodes, the paclitaxel concentrations were two to three times lower with liposome-encapsulated paclitaxel compared to those with conventional paclitaxel.[64] The significant decrease in toxicity and increase in plasma area under the curve and half-life with liposome-encapsulated paclitaxel indicate that this liposomal formulation may be a viable alternative to the conventional preparation of paclitaxel for therapeutic use.

### Paclitaxel Is First Liposomal Taxane to Enter Clinical Trials

Based on successful results obtained in preclinical studies of liposome-encapsulated paclitaxel, a phase I clinical trial was conducted in patients with advanced malignancies. Liposome-encapsulated paclitaxel is the first liposomal taxane to enter clinical trials.

Liposome-encapsulated paclitaxel was administered over 45 minutes every 3 weeks without antiemetics. To date, 26 patients have been treated at escalating dose levels: 3 patients at 90 mg/m², 3 at 135 mg/m², 11 at 175 mg/m², 6 at 250 mg/m², and 3 at 300 mg/m².

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A distinct toxicity profile was observed. Indeed, no clinically significant neuropathies or myalgias have been observed at the MTD level (175 mg/mg$^2$). Alopecia was also absent at the MTD or less. Dose-limiting toxicities included mucositis at 300 mg/m$^2$ (two patients); neutropenic sepsis, anaphylaxis at 250 mg/m$^2$ (two patients); and anaphylaxis at 175 mg/m$^2$ (one patient). Grade 4 neutropenia and leukopenia and grade 3 thrombocytopenia and anemia were first observed at the 175 mg/m$^2$ dose. Grade 3-4 mucositis and neutropenia were observed at liposome-encapsulated paclitaxel doses of 250 mg/m$^2$ or higher.

Liposome infusion reactions included transient back pain and flushing in five patients and rigors in one patient, but since routine premedication with diphenhydramine and hydrocortisone has been instituted, these effects have been mostly ameliorated. Two partial responses and three minor responses have been observed.[65]

## Conclusion and Implications

Liposomes are safe and effective drug carriers. Liposomal encapsulation of cytotoxic drugs diminishes toxicity to normal tissue while maintaining therapeutic efficacy. The pharmacokinetic and pharmacodynamic profiles of the free drug are favorably attenuated by liposomes. Through improvements and modifications of liposomal formulations, liposomes will continue to be important drug-delivery systems in oncology.


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# Real-time pharmacokinetics guiding clinical decisions: phase I study of a weekly schedule of liposome encapsulated paclitaxel in patients with solid tumours☆

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**Abstract**

The purpose of this weekly schedule phase I study of liposome encapsulated paclitaxel (LEP) was to define the maximum-tolerated dose (MTD), the recommended dose (RD), the dose-limiting toxicities (DLTs), the pharmacokinetic profiles, and to evaluate preliminarily antitumour effects in patients with refractory solid malignancies. LEP was administered as an intravenous (i.v.) infusion over 45 min once every week for 6 out of 8 weeks. Fourteen patients were treated at doses ranging from 90 to 150 mg/m$^2$/week. In one patient, DLT was observed at the dose level of 150 mg/m$^2$/week, who received less than 70% of the intended cumulative dose. No cumulative toxicities were observed. Stabilisation of disease for 8 weeks was documented in two patients. The whole blood clearance of total paclitaxel was similar for LEP (15.3±8.98 l/h/m$^2$) and Taxol® (17.5±3.43 l/h/m$^2$), and the extraliposomal to total drug ratio increased rapidly to unity at later sampling time points.. The trial was discontinued upon completion of enrolment of the 150 mg/m$^2$/week cohort because an assessment of the pharmacokinetics and clinical data suggested that LEP was unlikely to have any advantages over Taxol®. It is concluded that this formulation of LEP is unlikely to provide improvements over the taxanes currently in clinical use.



*Keywords:* Paclitaxel: Liposome: Drug delivery system: Phase I: Pharmacokinetics

## 1. Introduction

Paclitaxel, a complex diterpenoid natural product derived from the bark of the Western yew tree, *Taxus brevifolia*, belongs to the class of anti-microtubule agents and is active in a broad variety of human malignancies, including breast, ovarian and non-small cell lung cancer. Due to the agent's poor solubility in aqueous solutions, paclitaxel is formulated for clinical use in a mixture of Cremophor EL and ethanol (Taxol®). Previous work has indicated that Cremophor EL contributes to the non-linear pharmacokinetic behaviour of paclitaxel and to severe hypersensitivity reactions in humans observed after the administration of Taxol® [1,2]. The incidence of these severe hypersensitivity reactions is approximately 41% despite the use of premedication with corticosteroids and anti-histamines (see: http://www.taxol.com). It has been proposed that the hypersensitivity reaction to Taxol® is caused by a Cremophor EL-mediated activation of the complement system [3]. The clinical formulation of Taxol® has also been associated with other side-effects, including peripheral neurotoxicity [4].

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To overcome the problems associated with the current formulation of paclitaxel, several chemical, pharmaceutical, and/or biological strategies are being explored to optimise chemotherapeutic treatment with paclitaxel [5]. One of the strategies is delivery of the drug by the use of liposomes (ranging in size from 10 nm to 20 μm) consisting of an aqueous core surrounded by one or more membranes consisting of naturally or synthetic phospholipids arranged in a bilayer configuration [6,7]. These spherical vesicles can encapsulate various therapeutic agents, including anticancer agents [8]. One rationale for encapsulating cytotoxic drugs in liposomes is based on the hypothesis that macromolecular (liposomal) carrier leakage will occur in tumour tissue due to its enhanced permeability and retention (EPR) effect [9,10]. This EPR effect is caused by discontinuation of the endothelium of tumour blood vessels, as a result of structural and functional anomalies, and the co-existing lack of a fully functional system of lymphatic drainage [11]. The interplay between these characteristics of tumour tissue can result in the extravasation and retention of liposomes within the tumour interstitium, with the potential for providing more active drug to the tumour with less exposure to normal tissue.

Since liposome encapsulation is suitable for the intravenous (i.v.) delivery of poorly water-soluble compounds, paclitaxel has also been proposed for administration in liposomes [8]. A liposome formulation (without Cremophor EL) could have considerable potential given the problems associated with Cremophor EL. Several liposome-based formulations of paclitaxel have been tested *in vivo* for antitumour activity in various models [12–15], and for various liposome formulations the maximum tolerated dose (MTD) was 2- to 7-fold greater than for Taxol® [12].

The encapsulation of cytotoxic agents into liposomes (e.g. anthracyclines) has been shown to substantially modulate the pharmacokinetic behaviour of these drugs [16]. This approach may enhance the efficacy of anticancer drugs and reduce their systemic toxicity through the lower exposure of normal tissues to the drug. The aim of this study was to define the MTD, recommended dose (RD), dose-limiting toxicities (DLTs), pharmacokinetic profiles, and evaluate preliminarily antitumour effects of a weekly schedule of liposome encapsulated paclitaxel (LEP) in patients with refractory solid malignancies.

## 2. Patients and methods

### 2.1. Patient selection

Patients with a histologically-confirmed diagnosis of a malignant solid tumour refractory to conventional chemotherapy or for whom no effective therapy existed were eligible. Other eligibility criteria included the following: age $\geq 18$ years; Eastern Cooperative Oncology Group performance status $\leq 1$; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosourea or mitomycin-C); and adequate haematopoietic (absolute neutrophil count (ANC) $\geq 1.5\times10^9$ cells/l, platelet count $\geq 100\times10^9$ cells/l and haemoglobin $\geq 100$ g/l (or 6.2 mmol/l), hepatic (serum total bilirubin $\leq 25.65$ μmol/l, and serum aspartate transaminase (AST), alanine transaminase (ALT) $\leq 2.5$ times the institutional upper normal limit (UNL) ($\leq 5.0$ times UNL in case of liver metastases), and renal function (serum creatinine concentration $\leq 132.6$ μmol/l). Prior surgery or radiation therapy (irradiation field encompassing $<25\%$ of bone marrow) was acceptable as long as it had been completed at least 4 weeks before study registration. Specific exclusion criteria included known hypersensitivity to Cremophor EL and/or paclitaxel-containing regimens, known brain metastases, spinal cord compression, and/or carcinomatous meningitis. The study protocol was approved by the institutional Ethical Board, and all patients gave written informed consent before study entry.

### 2.2. Treatment and dose escalation

LEP was provided in vials containing 25 mg of paclitaxel per vial, and was supplied by Pharmacia (Nerviano, Italy) as a freeze-dried product. The vials also contained cardiolipin, egg phosphatidyl choline, cholesterol, D-α-tocopheryl acid succinate (vitamin E), and mannitol as inactive ingredients. The addition of mannitol as a cryoprotectant to this liposomal formulation of paclitaxel ensured that sonication before the administration of the drug was not required. The vials were stored at 5 °C in the dark, and were kept at room temperature for at least 2 h before reconstitution. After that, 25 ml of 0.9% sodium chloride injection per 25 mg of paclitaxel were added to the LEP vials. The solution was injected in the middle of the lyophilised cake using a 50 ml sterile and pyrogen-free syringe. The vials were gently shaken for 2–3 minutes. The reconstituted product was a sterile dispersion, and in-line filters were not used for administration. The content of the reconstituted vials was transferred to an infusion bag using a syringe, and the infusion bag was gently turned for 30 seconds before infusion.

LEP was given as a 45-min infusion, preceded by premedication consisting of 20 mg dexamethasone, 2 mg clemastine, and 50 mg ranitidine, each administered intravenously (i.v.) 30 min before the initiation of LEP infusion. Prophylactic anti-emetics were not given. Treatment was administered every 7 days for 6 out of 8 weeks, unless the patient did not recover adequately from treatment-related adverse events of the prior infusions. A period of 8 weeks was defined as one cycle. The

starting dose of LEP was 90 mg/m$^2$/week. This dose and schedule was selected on the basis of both clinical and pharmacokinetic data regarding weekly paclitaxel and from a phase I study of a sonicated preparation of LEP given as a single agent once every 3 weeks (unpublished data, Pharmacia). Subsequent dose levels scheduled were: 120 mg/m$^2$/week (33% dose increment), 150 mg/m$^2$/week (25%), and 180 mg/m$^2$/week (20%).

At least three patients were entered at each dose level. The MTD was defined as one dose level below the dose that induced DLTs during the first cycle in $\geq 2$ out of 6 patients. DLTs were defined using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 and included: grade 4 neutropenia $>7$ days, grade 4 haematological toxicity of any duration (except for grade 4 neutropenia), febrile neutropenia, non-haematological toxicities $\geq$grade 3, severe hypersensitivity reaction suggestive of an anaphylactic reaction, and receiving less than 70% of the intended cumulative dose of LEP [17]. If grade 2 neutropenia and/or grade 2 thrombocytopenia occurred during treatment, the dose of LEP was decreased by 50% for the subsequent administration. In case of grade $\geq 3$ neutropenia and/or thrombocytopenia, treatment with LEP was omitted for that week and then decreased by 50% for subsequent administrations when the neutrophil count had recovered to $\geq 1.5\times10^9$ cells/l and the platelet count to $\geq 75\times10^9$ cells/l. Intrapatient dose escalation was not allowed.

### 2.3. Treatment assessment

Before initiating therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell (CBC) count, including haematology tests (haemoglobin, white blood cells (WBC) with differential count, and platelets), and serum biochemistry, (sodium, potassium, calcium, magnesium, chloride, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, and gamma-glutamyltransferase) were performed, as were urinalysis (pH and albumin), electrocardiogram (ECG), and chest X-ray. Weekly evaluations included physical examination, toxicity assessment according to the NCI-CTC criteria, and haematology tests. Urinalysis was performed at week 1, 3 and 5 before the administration of LEP. Tumour evaluation was performed after every cycle of 8 weeks and response was assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST) [18]. Treatment was discontinued in cases of disease progression, unacceptable toxicity or patient request.

### 2.4. Sample collection and drug analysis

The pharmacokinetics of LEP were evaluated by following the time-concentration profile of paclitaxel, measured both as total paclitaxel in blood (liposome-associated plus non-liposome-associated) and extraliposomal paclitaxel in plasma (non-liposome-associated, bound and unbound to plasma proteins). The pharmacokinetics of extraliposomal and total paclitaxel after LEP administration were evaluated in all patients enrolled in the study during the first cycle of treatment. A total of 33 blood samples (approximately 7 ml) were drawn from each patient during the first and last week of the first cycle of treatment at pre-dose, end of infusion and at 5, 15, 30 min and 1, 2, 4 h, any time between 8 and 16 h, 24, 48, 72 and 168 h (this latter only at the sixth week) post-infusion. In addition, blood samples were also collected at pre-dose and the end of infusion at the second, third, fourth, and fifth weeks of treatment. Blood samples were collected in precooled (ice-water, 4 °C) vials containing lithium heparin as the anticoagulant. An aliquot of blood (2 ml) was frozen at −20 °C and used for the analysis of total paclitaxel; the remaining amount of blood was centrifuged (1200 *g* for 15 min at 4 °C) and the harvested plasma was frozen at −20 °C and used for the analysis of extraliposomal paclitaxel.

Concentrations of total paclitaxel (i.e. the sum of liposome-associated and non-liposome-associated paclitaxel) and extraliposomal paclitaxel (i.e. the non-liposome-associated, protein-bound and unbound) were determined in blood and plasma, respectively, with validated methods based on liquid chromatography with tandem mass-spectrometric detection (MS/MS). For the quantitation of total paclitaxel, Triton X-100 (5%, (v/v)) was added to whole blood and an aliquot (100 μl) of blood was extracted using *tert*-butyl methyl ether (MTBE). To determine extraliposomal concentrations of paclitaxel in human plasma (250 μl), liposomes were separated from plasma proteins using a solution containing dodecantungstophosphoric acid and magnesium chloride; an aliquot of supernatant (100 μl) was extracted with MTBE. For both methods, paclitaxel was separated using a Zorbax C18 column and eluted under gradient conditions with a mobile phase containing acetonitrile and 2 mM ammonium acetate buffer (pH 5). MS/MS detection was conducted with a PE-Sciex API 3000 mass spectrometer using a turbo ionspray source and multiple reaction monitoring in a positive ion mode. The lower limits of quantitation were 5 ng/ml and 1 ng/ml for total paclitaxel in blood and extraliposomal in plasma, respectively.

Analysis of the protein-unbound fraction of the extraliposomal paclitaxel concentrations was attempted, but could not be determined separately due to interference in the equilibrium dialysis method [19].

### 2.5. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by standard non-compartmental methods using the WinNonlin

version 3.1 (Pharsight, Mountain View, CA, USA). The area under the plasma concentration-time curve (AUC) was calculated up to the last detectable concentration ($C_{(t,z)}$), using the linear trapezoidal rule. Other parameters, including volume of distribution at steady state ($V_{ss}$) and clearance (CL) were estimated using standard equations. The AUC was extrapolated to infinity by the addition of $C_{(t,z)}/k$, where k is the terminal rate constant, which was estimated from a log-linear regression analysis of the terminal disposition phase. The half-life of the terminal phase was calculated as ln(2)/k.

## 3. Results

A total of 14 patients were entered onto the study and received at least one dose of LEP. All patients were assessable for toxicity and response. Patients' characteristics are listed in Table 1. Four patients did not complete the first cycle; one patient with adenocarcinoma of the duodenum, developed tumour-related haematemesis and three patients had disease progression. The total number of assessable cycles was 16. The median number of cycles per patient was 1 (range, 1–2).

Table 1
Patients' characteristics

| Characteristic | No. of patients |
|---|---|
| No. of patients | |
| Total | 14 |
| Assessment | |
| For dose-limiting toxicity | 13 |
| For efficacy | 12 |
| No. of cycles/patient | |
| Median (range) | 1 (1–2) |
| Gender | |
| Male | 8 |
| Female | 6 |
| Age (years) | |
| Median (range) | 54 (30–66) |
| ECOG performance status | |
| 0 | 4 |
| 1 | 10 |
| Previous therapy | |
| Chemotherapy only | 11 |
| Radiotherapy | 3 |
| Surgery | 9 |
| Tumour types | |
| Colorectal | 3 |
| Gastro-intestinal tract, including: | 8 |
| Esophageal | 1 |
| Gastric | 2 |
| Gallbladder | 1 |
| Pancreatic | 2 |
| Unknown primary tumour | 2 |
| Miscellaneous | 3 |

ECOG, Eastern Cooperative Oncology Group.

### 3.1. Dose-limiting toxicity

At 150 mg/m$^2$/week, one patient experienced DLT by receiving less than 70% of the intended cumulative dose of LEP. The patient received the first two doses as planned. The third and fourth infusions were omitted because of grade 3 and grade 4 neutropenia, respectively, and the patient received no subsequent therapy because of disease progression. In view of this single DLT, the cohort was expanded without observing further DLTs. Dose escalation to 180 mg/m$^2$/week did not take place because of discontinuation of the study (see below). For this reason, the MTD and RD were not determined.

### 3.2. Haematological toxicity

Haematological toxicities per patient over the entire course of treatment are summarised in Table 2. One patient experienced grade 3 neutropenia at the 120 mg/m$^2$/week dose level and one patient at the 150 mg/m$^2$/week dose level experienced grade 3 and grade 4 neutropenia. At the 150 mg/m$^2$/week dose level, haematological side-effects generally lasted less than 7 days. Mild to moderate anaemia and thrombocytopenia were documented at all dose levels tested.

### 3.3. Non-haematological toxicity

Gastrointestinal toxicities of mild to moderate severity were observed at all dose levels tested, and consisted of nausea [grade 1 ($N=7$), grade 2 ($N=1$), grade 3 ($N=1$)], diarrhoea [grade 1 ($N=8$), grade 2 ($N=1$), grade 3 ($N=1$)], and vomiting [grade 1 ($N=3$)]. Mild hypersensitivity reactions, consisting mainly of a facial flush and shortness of breath, were documented in four patients at the 150 mg/m$^2$/week dose level. All of these reactions had a rapid onset within the first minutes after the start of the LEP infusion and promptly recovered after stopping of the infusion and i.v. administration of 2 mg clemastine and 100 mg hydrocortisone. After rechallenging, none of these patients experienced a repeat of the infusion reaction. In two patients, a transient grade 1 to 2 skin reaction was documented. Mild alopecia (grade 1) was observed in 1 patient and neurotoxicity was not observed, but particularly the latter should be interpreted with caution in view of the very small number of cycles evaluated per patient.

### 3.4. Pharmacokinetics

Pharmacokinetic analysis was performed on all 14 patients enrolled in this study. The pharmacokinetic parameters for total and extraliposomal paclitaxel during the first and sixth week of the LEP treatment are summarised in Tables 3 and 4. After the administration

of LEP at dose levels 90, 120, and 150 mg/m$^2$/week, the levels of total and extraliposomal paclitaxel reached the maximum value near the end of infusion on both the first and sixth week of treatment. After the administration of 90, 120 and 150 mg/m$^2$/week, the mean (±S.D.) peak concentration of extraliposomal paclitaxel was 190±94 and 186±70 ng/ml, 363±241 and 224 ng/ml, and 424±166 and 326±120 ng/ml, after the first and sixth week, respectively. The corresponding values of total paclitaxel were 2787±1262 and 3083±807 ng/ml, 3918±1325 ng/ml and 2020 ng/ml, and 5004±2334 and 5032±3527 ng/ml, after the first and sixth week, respectively, at the 90, 120 and 150 mg/m$^2$/week dose levels. After the end of infusion, blood levels of total paclitaxel and plasma levels of extraliposomal paclitaxel declined polyexponentially with an apparent terminal half-life ranging between 77 and 195 h, and 80 and 144 h, respectively. Total paclitaxel exhibited a relatively slow clearance from whole blood (range, 9 to 26 l/h/m$^2$), with a steady-state volume of distribution ranging from 120 to 2189 l/m$^2$. There was large interpatient variation in both drug clearance and volume of distribution at a coefficient of variation of approximately 60%.

After the first week of treatment, the levels of total and extraliposomal paclitaxel increased in direct proportion with the dose, suggesting linear pharmacokinetics. Furthermore, over the tested dose range, total blood clearance was dose-independent ($P=0.490$,

Table 2
Haematological toxicity (worst grade per patient (pt))

| | | | Grades | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Anaemia | | Leucocytopenia | | | Neutropenia | | | Thrombocytopenia | | |
| Dose (mg/m$^2$/week) | No. of pts. | No. of cycles | 1–2 | 3–4 | 1–2 | 3 | 4 | 1–2 | 3 | 4 | 1–2 | 3 | 4 |
| 90 | 3 | 3 | 3 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 120 | 4 | 4 | 2 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 |
| 150 | 7 | 9 | 5 | 0 | 3 | 3 | 0 | 3 | 1 | 1 | 2 | 0 | 0 |

Table 3
Mean±S.D. plasma pharmacokinetic parameters of total paclitaxel during the 1st week and 6th week of LEP treatment

| Dose (mg/m$^2$/week) | No. of patients | 1st week | | | | | | 6th week | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_{max}$ (ng/ml) | $T_{1/2,z}$ (h) | $AUC_{0-tlast}$ (ng·h/ml) | $AUC_{0-\infty}$ (ng·h/ml) | CL (l/h/m$^2$) | $V_{ss}$ (l/m$^2$) | No. of patients | $C_{max}$ (ng/ml) | $T_{1/2,z}$ (h) | $AUC_{0-tlast}$ (ng·h/ml) |
| 90 | 3 | 2787±1262 | 135±67 | 6444±12 | 8035±1111 | 11±2 | 1004±506 | 3 | 3083±807 | 195[a]±61 | 12494±5194 |
| 120 | 4 | 3918±1325 | 77±68[a] | 6566±3231 | 7611±2492 | 17±5 | 618±316 | 1 | 2020 | 112 | 5731 |
| 150 | 7 | 5004±2334 | 118±83 | 10609±2852 | 12539±3260 | 13±6 | 1036±824 | 5 | 5032±3527 | 145[b]±125 | 13759±5143 |

$C_{max}$, peak blood concentration; $T_{1/2,z}$, half life of the terminal disposition phase; $AUC_{0-tlast}$, area under the blood concentration-time curve up to the last time point with measurable levels; $AUC_{0-\infty}$, AUC extrapolated to infinity; CL, total blood clearance; $V_{ss}$, steady-state volume of distribution.

[a] $N=2$.

[b] $N=3$.

Table 4
Mean±SD plasma pharmacokinetic parameters of extraliposomal (protein-bound) paclitaxel during the 1st week and 6th week of LEP treatment

| Dose (mg/m$^2$/week) | No. of patients | 1st week | | | | 6th week | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $C_{max}$ (ng/ml) | $T_{1/2,z}$ (h) | $AUC_{0-tlast}$ (ng·h/ml) | $AUC_{0-\infty}$ (ng·h/ml) | No. of patients | $C_{max}$ (ng/ml) | $T_{1/2,z}$ (h) | $AUC_{0-t\ (last)}$ (ng·h/ml) |
| 90 | 3 | 190±94 | 80[a] | 910±171 | N/A | 3 | 186±70 | 135±67 | 4242±2658 |
| 120 | 4 | 363±241 | 138[b]±74 | 1357±287 | 2119±576 | 1 | 224[a] | N/A | 2826 |
| 150 | 7 | 424±166 | 126[b]±32 | 2410±704 | 3031±839 | 5 | 326±120 | 144[c]±48 | 3967±1321 |

$C_{max}$, peak blood concentration; $T_{1/2,z}$, half life of the terminal disposition phase; $AUC_{0-tlast}$, area under the blood concentration-time curve up to the last time point with measurable levels; $AUC_{0-\infty}$, AUC extrapolated to infinity; N/A not available.

[a] $N=1$.

[b] $N=3$.

[c] $N=2$.

Kruskal–Wallis one-way ANOVA, corrected for ties), supporting the above observation of a linear kinetics for total paclitaxel. Pharmacokinetic information obtained during the sixth week indicated on average an one- to two-fold accumulation, which is in reasonable agreement with the half-life of the apparent terminal disposition phase.

Assuming an equal distribution between plasma and blood for extraliposomal paclitaxel following the administration of LEP [20], extraliposomal paclitaxel represented only a minor portion of the total paclitaxel (i.e. the sum of liposome-associated and non-liposome-associated paclitaxel) in the systemic circulation. The proportion of extraliposomal and total paclitaxel changed with time and among patients, ranging from 3 to 14% of the total paclitaxel at the first time point to approximately 23–100% at the final sampling time points. On average, considering the overall exposure, extraliposomal paclitaxel in the plasma accounted for approximately 14–49% of the total paclitaxel exposure. The mean extraliposomal paclitaxel plasma concentrations and the total paclitaxel concentrations in blood versus time curves observed at the 150 mg/m$^2$/week dose level are displayed in Figs. 1 and 2.

### 3.5. Antitumour efficacy

At the 150 mg/m$^2$/week dose level, disease stabilisation for 8 weeks was documented in two patients with liver metastases of an adenocarcinoma of unknown primary and with pleural and peritoneal metastatic oesophageal carcinoma, respectively. The other patients had progressive disease after the tumour assessment at 8 weeks, with the exception of 2 patients who discontinued treatment because of early progression after the first and fourth administrations of LEP, respectively.

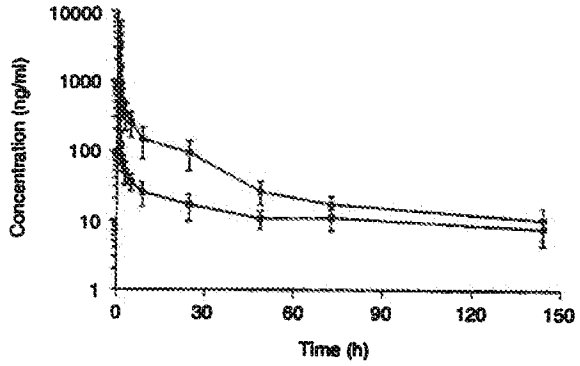


Fig. 1. Plasma concentration-time profiles of total paclitaxel in whole blood (closed symbols) and extraliposomal paclitaxel in plasma (open symbols) in patients receiving liposome encapsulated paclitaxel (LEP) at a dose of 150 mg/m$^2$/week. Data are presented as mean values (symbol)±standard deviation (S.D.).

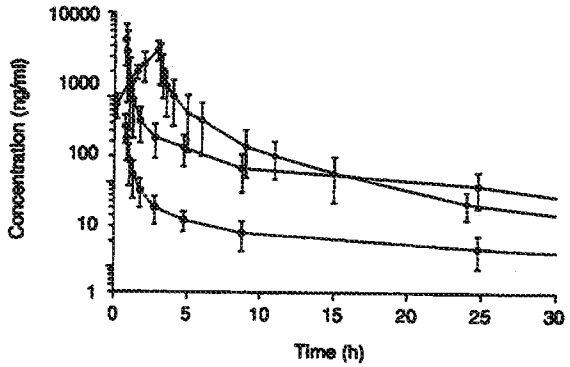


Fig. 2. Plasma concentration-time profiles of total paclitaxel in whole blood (closed symbols). extraliposomal paclitaxel in plasma (open symbols) in patients receiving liposome encapsulated paclitaxel LEP at a dose of 150 mg/m$^2$/week, and total paclitaxel in plasma (open lozenges) in 14 patients receiving Taxol® at a dose of 150 mg/m$^2$ (3-h infusion). Data are presented as mean values (symbol)±standard deviation (S.D.).

## 4. Discussion

The current study was performed to explore the safety, feasibility, and pharmacokinetics of a new liposomal formulation of paclitaxel administrated as a 45 min i.v. infusion once weekly for 6 out of 8 consecutive weeks. A previous formulation requiring a cumbersome sonication step just prior to infusion had been tested in a phase I trial using a single dose given every 21 days. The new, freeze-dried formulation has the same composition as the previously tested LEP formulation with the exception of the addition of mannitol as a cryoprotectant. The non-sonicated formulation was developed with the objectives of improving the overall profile of the drug. Real-time pharmacokinetics complimented the clinical findings indicating that LEP was unlikely to offer significant advantages over the taxanes currently employed in the clinic. With the new formulation, myelosuppression was seen at the 150 mg/m$^2$/week dose level, suggesting that a possible recommended phase II dose using a weekly schedule would be in the range of 150–180 mg/m$^2$/week. However, the current results do not allow any conclusions regarding long-term or cumulative toxicity, including neurotoxicity.

To be effective as a carrier, a liposome must be able to efficiently balance stability in the circulation with the ability to make the drug available at the tumour [8]. In order to achieve the optimum efficacy for a drug-delivery system, it is necessary to encapsulate the maximum possible quantity of a drug [7]. Comparison of the encapsulation efficiency of the drug in liposomes with the therapeutic dose indicates whether liposomes can be used as a suitable drug-delivery system [7]. The retention of the encapsulated drug is determined by the physicochemical characteristics of the drug itself and by the lipid composition and number of concentric membranes

of the liposomal vesicle [21,22]. Highly hydrophobic drugs, like paclitaxel, tend to associate mainly with the bilayer compartment of the liposome, resulting in lower entrapment stability due to faster redistribution of the drug to plasma components [9].

In comparison with small molecules, the volume of distribution of the drug encapsulated in liposomes is usually significantly reduced [9], and when a drug is stably encapsulated within the liposomal matrix it displays the pharmacokinetic profile of the intact liposome rather than that of the encapsulated agent [11]. In general, this should achieve a significant increase in the AUC in the circulation, and possibly in tumour tissue, and mimick the effect of administering cytotoxic drugs as a continuous i.v.infusion, without the inconvience of i.v. devices and toxicities associated with systemic drug exposure [11]. Likewise, the clearance of anticancer drugs encapsulated in liposomes is usually reduced, and the elimination half-life prolonged [23], as has been shown previously for anthracyclines [24], vincristine [25] and lurtotecan [26].

The pharmacokinetics of total paclitaxel when administered as LEP appeared to be dose-independent, providing further evidence of the previous supposition that the non-linearity of paclitaxel disposition following the administration of Taxol® is caused by its excipient [1]. However, as compared with Taxol®, the interpatient variation in paclitaxel pharmacokinetic parameters following the administration of LEP was large (up to 60%). In contrast to that expected for a liposomal formulation, the clearance of total paclitaxel in whole blood following LEP ($15.3 \pm 8.98$ l/h/m$^2$) was very similar to that reported after administration of Taxol® ($17.5 \pm 3.43$ l/h/m$^2$) [27]. The observed values for volume of distribution at steady state of total paclitaxel in patients receiving LEP was very high (approximately 1000 l/m$^2$). Furthermore, in the systemic circulation, most paclitaxel was associated with the liposomes, since the extraliposomal paclitaxel AUC accounted only for 14–49% of the total paclitaxel AUC; however, the proportion of extraliposomal drug in plasma and total drug in whole blood increased with the time, reaching unity at the end of the sampling time period.

Previous work has shown that the plasma protein binding of the fraction of unbound paclitaxel in plasma in the absence of formulation excipients is approximately 85% in humans [1]. Assuming this value of plasma protein binding also for paclitaxel after LEP administration, at the 150 mg/m$^2$/week dose level, the predicted $AUC_{0-t(last)}$ is $362 \pm 106$ ng·h/ml, which is similar to $397 \pm 69.7$ ng·h/ml observed for Taxol® at the recommended weekly dose of 100 mg/m$^2$ [28]. This clearly suggests that at approximate equitoxic doses, exposure to the clinical relevant pharmacokinetic parameter is comparable for both formulations, and that LEP provides no pharmacological advantages over Taxol®.

The release of complement fragments C3a and C5a can cause a hypersensitivity reaction, called complement activation-related pseudoallergy (CARPA), by release of anaphylatoxins and a cascade of cellular mediators of inflammation [3,29,30]. Activation of complement can rapidly induce several symptoms, including pain (e.g. chest-pain, low back pain, headache), chills, choking, nausea, confusion, skin toxicity (e.g. erythema, pruritus, urticaria), symptoms of respiratory distress (e.g. bronchospasm, dyspnoea), and severe cardiac arrhythmias [3,29]. The frequency of CARPA due to i.v. infusion of conventional or pegylated liposomes ranged from 3 to 7% in several studies. Symptoms were observed within 5 to 10 min after the start of the first infusion. In most patients, symptoms disappeared shortly after stopping of the infusion [3]. The rapid induction of this event seems to indicate that minimal amounts of liposomes can induce these side-effects [30]. In the present study, hypersensitivity reactions were observed shortly after the i.v. infusion of the first LEP treatment in 3 patients. Rechallenge of the LEP infusion after treatment of corticosteroids and antihistamines was possible in all 3 patients without any new reaction or other complications. While the frequency of reactions was relatively low, LEP did not distinguish itself from currently used taxanes, since all patients received standard premedication with dexamethasone and antihistamines.

Collectively, the results of pharmacokinetic data supported the decision to terminate the study prior to reaching the primary objective of determining the MTD and RD and strengthened the importance of performing a real-time pharmacokinetic analysis during a phase I study in order to bring as much as relevant information as possible to guide future clinical treatment decisions.

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# Oncology Tools: Dose Calculator

This calculator will convert a dose in mg/kg or mg/m2 to a total dose. It will also indicate the equivalent dose for several species of animals. The method to convert a dose from one species to another is to set the dose in mg/m2 the same. As an example, a mouse dose of 50 mg/kg is equivalent to a dose of about 150 mg/m2. To find an equivalent human dose, set the dose to 150 mg/m2 and then read that the equivalent human dose is about 3.5 mg/kg. The weight in kilograms can be adjusted to any value to individualize dosing. The basis for the calculations is the formula mg/m5 = (mg/kg x 100 x kga)/K where K is a different value for each animal species. The weights are entered in kilograms. The estimated surface area is based on the formula BSA = (K x kgb)/100 where K is the same K value as in the previous equation and a different value for each animal species

---

1. **Select Dosage Units**: ○ mg/kg ◉ mg/m2
2. **Enter Dosage Value**: 50
3. If you wish to modify the default values for **Human** weight and/or height, enter the desired changes below:

**Enter Weight** 70 in: ◉ kilograms or ○ pounds

**Enter Height** 68 in: ○ centimeters or ◉ inches

Calculate

4. If you wish to modify the default weights (in kilograms) for animal species shown to the right and below, enter desired weight below:

| | | | |
|---|---|---|---|
| Mouse: | 0.02 | Rabbit: | 2.00 |
| Hamster: | 0.03 | Cat: | 2.50 |
| Rat: | 0.15 | Monkey: | 3.00 |
| Guinea Pig: | 1.0 | Dog: | 8.00 |

Calculate Reset Defaults

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# Oncology Tools: Dose Calculator

## Dose Calculator Results

Please note that for regulatory submissions the FDA recommends the following conversion factors: Mouse = 3, Hamster = 4.1, Rat = 6, Guinea Pig = 7.7. (based on Cancer Chemother Repts 50(4):219(1966)) Multiply the conversion factor by the animal dose in mg/kg to obtain the dose in mg/m2 for human dose equivalent. when both height and weight are known, human body surface area is calculated using Boyd's Formula of Body Surface Area (Boyd E. The growth of the surface area of the human body. University of Minnesota Press. 1935) . Calculations with weight alone (no heigth) are less accurate. All values are estimates and values above 2.25 m2 are not considered accurate.

| Species | Weight, kg | Est. Total Dose, mg | Dose in mg/kg | Dose in mg/m2 | Est. BSA,m2 |
|---|---|---|---|---|---|
| Human | 70.00 | 92.17 | 1.28 | 50.00 | 1.843 |
| Mouse | 0.02 | 0.33 | 16.58 | 50.00 | 0.007 |
| Hamster | 0.03 | 0.43 | 14.47 | 50.00 | 0.009 |
| Rat | 0.15 | 1.27 | 8.47 | 50.00 | 0.025 |
| Guinea Pig | 1.00 | 4.45 | 4.45 | 50.00 | 0.089 |
| Rabbit | 2.00 | 7.94 | 3.97 | 50.00 | 0.159 |
| Cat | 2.50 | 9.85 | 3.94 | 50.00 | 0.197 |
| Monkey | 3.00 | 12.27 | 4.09 | 50.00 | 0.245 |
| Dog | 8.00 | 22.40 | 2.80 | 50.00 | 0.448 |

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# Oncology Tools: Dose Calculator

This calculator will convert a dose in mg/kg or mg/m2 to a total dose. It will also indicate the equivalent dose for several species of animals. The method to convert a dose from one species to another is to set the dose in mg/m2 the same. As an example, a mouse dose of 50 mg/kg is equivalent to a dose of about 150 mg/m2. To find an equivalent human dose, set the dose to 150 mg/m2 and then read that the equivalent human dose is about 3.5 mg/kg. The weight in kilograms can be adjusted to any value to individualize dosing. The basis for the calculations is the formula mg/m5 = (mg/kg x 100 x kga)/K where K is a different value for each animal species. The weights are entered in kilograms. The estimated surface area is based on the formula BSA = (K x kgb)/100 where K is the same K value as in the previous equation and a different value for each animal species

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1. **Select Dosage Units**: ○ mg/kg ◉ mg/m2
2. **Enter Dosage Value**: 75
3. If you wish to modify the default values for **Human** weight and/or height, enter the desired changes below:

**Enter Weight** 70 in: ◉ kilograms or ○ pounds

**Enter Height** 68 in: ○ centimeters or ◉ inches

Calculate

4. If you wish to modify the default weights (in kilograms) for animal species shown to the right and below, enter desired weight below:

| | | | |
|---|---|---|---|
| Mouse: | 0.02 | Rabbit: | 2.00 |
| Hamster: | 0.03 | Cat: | 2.50 |
| Rat: | 0.15 | Monkey: | 3.00 |
| Guinea Pig: | 1.0 | Dog: | 8.00 |

Calculate Reset Defaults

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# Oncology Tools: Dose Calculator

## Dose Calculator Results

Please note that for regulatory submissions the FDA recommends the following conversion factors: Mouse = 3, Hamster = 4.1, Rat = 6, Guinea Pig = 7.7. (based on Cancer Chemother Repts 50(4):219(1966)) Multiply the conversion factor by the animal dose in mg/kg to obtain the dose in mg/m2 for human dose equivalent. when both height and weight are known, human body surface area is calculated using Boyd's Formula of Body Surface Area (Boyd E. The growth of the surface area of the human body. University of Minnesota Press. 1935) . Calculations with weight alone (no heigth) are less accurate. All values are estimates and values above 2.25 m2 are not considered accurate.

| Species | Weight, kg | Est. Total Dose, mg | Dose in mg/kg | Dose in mg/m2 | Est. BSA,m2 |
|---|---|---|---|---|---|
| Human | 70.00 | 138.26 | 1.93 | 75.00 | 1.843 |
| Mouse | 0.02 | 0.50 | 24.87 | 75.00 | 0.007 |
| Hamster | 0.03 | 0.65 | 21.70 | 75.00 | 0.009 |
| Rat | 0.15 | 1.91 | 12.70 | 75.00 | 0.025 |
| Guinea Pig | 1.00 | 6.68 | 6.68 | 75.00 | 0.089 |
| Rabbit | 2.00 | 11.91 | 5.95 | 75.00 | 0.159 |
| Cat | 2.50 | 14.78 | 5.91 | 75.00 | 0.197 |
| Monkey | 3.00 | 18.41 | 6.14 | 75.00 | 0.245 |
| Dog | 8.00 | 33.60 | 4.20 | 75.00 | 0.448 |

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# Oncology Tools: Dose Calculator

This calculator will convert a dose in mg/kg or mg/m2 to a total dose. It will also indicate the equivalent dose for several species of animals. The method to convert a dose from one species to another is to set the dose in mg/m2 the same. As an example, a mouse dose of 50 mg/kg is equivalent to a dose of about 150 mg/m2. To find an equivalent human dose, set the dose to 150 mg/m2 and then read that the equivalent human dose is about 3.5 mg/kg. The weight in kilograms can be adjusted to any value to individualize dosing. The basis for the calculations is the formula mg/m5 = (mg/kg x 100 x kga)/K where K is a different value for each animal species. The weights are entered in kilograms. The estimated surface area is based on the formula BSA = (K x kgb)/100 where K is the same K value as in the previous equation and a different value for each animal species

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1. **Select Dosage Units**: ○ mg/kg ◉ mg/m2
2. **Enter Dosage Value**: 90
3. If you wish to modify the default values for **Human** weight and/or height, enter the desired changes below:

**Enter Weight** 70 in: ◉ kilograms or ○ pounds

**Enter Height** 68 in: ○ centimeters or ◉ inches

Calculate

4. If you wish to modify the default weights (in kilograms) for animal species shown to the right and below, enter desired weight below:

| | | | |
|---|---|---|---|
| Mouse: | 0.02 | Rabbit: | 2.00 |
| Hamster: | 0.03 | Cat: | 2.50 |
| Rat: | 0.15 | Monkey: | 3.00 |
| Guinea Pig: | 1.0 | Dog: | 8.00 |

Calculate Reset Defaults

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# Oncology Tools: Dose Calculator

## Dose Calculator Results

Please note that for regulatory submissions the FDA recommends the following conversion factors: Mouse = 3, Hamster = 4.1, Rat = 6, Guinea Pig = 7.7. (based on Cancer Chemother Repts 50(4):219(1966)) Multiply the conversion factor by the animal dose in mg/kg to obtain the dose in mg/m2 for human dose equivalent. when both height and weight are known, human body surface area is calculated using Boyd's Formula of Body Surface Area (Boyd E. The growth of the surface area of the human body. University of Minnesota Press. 1935) . Calculations with weight alone (no heigth) are less accurate. All values are estimates and values above 2.25 m2 are not considered accurate.

| Species | Weight, kg | Est. Total Dose, mg | Dose in mg/kg | Dose in mg/m2 | Est. BSA,m2 |
|---|---|---|---|---|---|
| Human | 70.00 | 165.91 | 2.31 | 90.00 | 1.843 |
| Mouse | 0.02 | 0.60 | 29.84 | 90.00 | 0.007 |
| Hamster | 0.03 | 0.78 | 26.04 | 90.00 | 0.009 |
| Rat | 0.15 | 2.29 | 15.24 | 90.00 | 0.025 |
| Guinea Pig | 1.00 | 8.01 | 8.01 | 90.00 | 0.089 |
| Rabbit | 2.00 | 14.29 | 7.14 | 90.00 | 0.159 |
| Cat | 2.50 | 17.74 | 7.10 | 90.00 | 0.197 |
| Monkey | 3.00 | 22.09 | 7.36 | 90.00 | 0.245 |
| Dog | 8.00 | 40.32 | 5.04 | 90.00 | 0.448 |

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# Oncology Tools: Dose Calculator

This calculator will convert a dose in mg/kg or mg/m2 to a total dose. It will also indicate the equivalent dose for several species of animals. The method to convert a dose from one species to another is to set the dose in mg/m2 the same. As an example, a mouse dose of 50 mg/kg is equivalent to a dose of about 150 mg/m2. To find an equivalent human dose, set the dose to 150 mg/m2 and then read that the equivalent human dose is about 3.5 mg/kg. The weight in kilograms can be adjusted to any value to individualize dosing. The basis for the calculations is the formula mg/m5 = (mg/kg x 100 x kga)/K where K is a different value for each animal species. The weights are entered in kilograms. The estimated surface area is based on the formula BSA = (K x kgb)/100 where K is the same K value as in the previous equation and a different value for each animal species

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1. **Select Dosage Units**: ○ mg/kg ◉ mg/m2
2. **Enter Dosage Value**: 135
3. If you wish to modify the default values for **Human** weight and/or height, enter the desired changes below:

**Enter Weight** 70 in: ◉ kilograms or ○ pounds

**Enter Height** 68 in: ○ centimeters or ◉ inches

Calculate

4. If you wish to modify the default weights (in kilograms) for animal species shown to the right and below, enter desired weight below:

| | | | |
|---|---|---|---|
| Mouse: | 0.02 | Rabbit: | 2.00 |
| Hamster: | 0.03 | Cat: | 2.50 |
| Rat: | 0.15 | Monkey: | 3.00 |
| Guinea Pig: | 1.0 | Dog: | 8.00 |

Calculate Reset Defaults

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# Oncology Tools: Dose Calculator

## Dose Calculator Results

Please note that for regulatory submissions the FDA recommends the following conversion factors: Mouse = 3, Hamster = 4.1, Rat = 6, Guinea Pig = 7.7. (based on Cancer Chemother Repts 50(4):219(1966)) Multiply the conversion factor by the animal dose in mg/kg to obtain the dose in mg/m2 for human dose equivalent. when both height and weight are known, human body surface area is calculated using Boyd's Formula of Body Surface Area (Boyd E. The growth of the surface area of the human body. University of Minnesota Press. 1935) . Calculations with weight alone (no heigth) are less accurate. All values are estimates and values above 2.25 m2 are not considered accurate.

| Species | Weight, kg | Est. Total Dose, mg | Dose in mg/kg | Dose in mg/m2 | Est. BSA,m2 |
|---|---|---|---|---|---|
| Human | 70.00 | 248.86 | 3.47 | 135.00 | 1.843 |
| Mouse | 0.02 | 0.90 | 44.76 | 135.00 | 0.007 |
| Hamster | 0.03 | 1.17 | 39.06 | 135.00 | 0.009 |
| Rat | 0.15 | 3.43 | 22.87 | 135.00 | 0.025 |
| Guinea Pig | 1.00 | 12.02 | 12.02 | 135.00 | 0.089 |
| Rabbit | 2.00 | 21.43 | 10.71 | 135.00 | 0.159 |
| Cat | 2.50 | 26.61 | 10.64 | 135.00 | 0.197 |
| Monkey | 3.00 | 33.14 | 11.05 | 135.00 | 0.245 |
| Dog | 8.00 | 60.48 | 7.56 | 135.00 | 0.448 |

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# Oncology Tools: Dose Calculator

This calculator will convert a dose in mg/kg or mg/m2 to a total dose. It will also indicate the equivalent dose for several species of animals. The method to convert a dose from one species to another is to set the dose in mg/m2 the same. As an example, a mouse dose of 50 mg/kg is equivalent to a dose of about 150 mg/m2. To find an equivalent human dose, set the dose to 150 mg/m2 and then read that the equivalent human dose is about 3.5 mg/kg. The weight in kilograms can be adjusted to any value to individualize dosing. The basis for the calculations is the formula mg/m5 = (mg/kg x 100 x kga)/K where K is a different value for each animal species. The weights are entered in kilograms. The estimated surface area is based on the formula BSA = (K x kgb)/100 where K is the same K value as in the previous equation and a different value for each animal species

1. **Select Dosage Units**: ○ mg/kg ◉ mg/m2
2. **Enter Dosage Value**: 175
3. If you wish to modify the default values for **Human** weight and/or height, enter the desired changes below:

**Enter Weight** 70 in: ◉ kilograms or ○ pounds

**Enter Height** 68 in: ○ centimeters or ◉ inches

Calculate

4. If you wish to modify the default weights (in kilograms) for animal species shown to the right and below, enter desired weight below:

| | | | |
|---|---|---|---|
| Mouse: | 0.02 | Rabbit: | 2.00 |
| Hamster: | 0.03 | Cat: | 2.50 |
| Rat: | 0.15 | Monkey: | 3.00 |
| Guinea Pig: | 1.0 | Dog: | 8.00 |

Calculate Reset Defaults

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# Oncology Tools: Dose Calculator

## Dose Calculator Results

Please note that for regulatory submissions the FDA recommends the following conversion factors: Mouse = 3, Hamster = 4.1, Rat = 6, Guinea Pig = 7.7. (based on Cancer Chemother Repts 50(4):219(1966)) Multiply the conversion factor by the animal dose in mg/kg to obtain the dose in mg/m2 for human dose equivalent. when both height and weight are known, human body surface area is calculated using Boyd's Formula of Body Surface Area (Boyd E. The growth of the surface area of the human body. University of Minnesota Press. 1935) . Calculations with weight alone (no heigth) are less accurate. All values are estimates and values above 2.25 m2 are not considered accurate.

| Species | Weight, kg | Est. Total Dose, mg | Dose in mg/kg | Dose in mg/m2 | Est. BSA,m2 |
|---|---|---|---|---|---|
| Human | 70.00 | 322.60 | 4.49 | 175.00 | 1.843 |
| Mouse | 0.02 | 1.16 | 58.02 | 175.00 | 0.007 |
| Hamster | 0.03 | 1.52 | 50.63 | 175.00 | 0.009 |
| Rat | 0.15 | 4.45 | 29.64 | 175.00 | 0.025 |
| Guinea Pig | 1.00 | 15.58 | 15.58 | 175.00 | 0.089 |
| Rabbit | 2.00 | 27.78 | 13.89 | 175.00 | 0.159 |
| Cat | 2.50 | 34.49 | 13.80 | 175.00 | 0.197 |
| Monkey | 3.00 | 42.95 | 14.32 | 175.00 | 0.245 |
| Dog | 8.00 | 78.40 | 9.80 | 175.00 | 0.448 |

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# Oncology Tools: Dose Calculator

This calculator will convert a dose in mg/kg or mg/m2 to a total dose. It will also indicate the equivalent dose for several species of animals. The method to convert a dose from one species to another is to set the dose in mg/m2 the same. As an example, a mouse dose of 50 mg/kg is equivalent to a dose of about 150 mg/m2. To find an equivalent human dose, set the dose to 150 mg/m2 and then read that the equivalent human dose is about 3.5 mg/kg. The weight in kilograms can be adjusted to any value to individualize dosing. The basis for the calculations is the formula mg/m5 = (mg/kg x 100 x kga)/K where K is a different value for each animal species. The weights are entered in kilograms. The estimated surface area is based on the formula BSA = (K x kgb)/100 where K is the same K value as in the previous equation and a different value for each animal species

---

1. **Select Dosage Units**: ○ mg/kg ◉ mg/m2
2. **Enter Dosage Value**: 300
3. If you wish to modify the default values for **Human** weight and/or height, enter the desired changes below:

**Enter Weight** 70 in: ◉ kilograms or ○ pounds

**Enter Height** 68 in: ○ centimeters or ◉ inches

Calculate

4. If you wish to modify the default weights (in kilograms) for animal species shown to the right and below, enter desired weight below:

| | | | |
|---|---|---|---|
| Mouse: | 0.02 | Rabbit: | 2.00 |
| Hamster: | 0.03 | Cat: | 2.50 |
| Rat: | 0.15 | Monkey: | 3.00 |
| Guinea Pig: | 1.0 | Dog: | 8.00 |

Calculate Reset Defaults

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# Oncology Tools: Dose Calculator

## Dose Calculator Results

Please note that for regulatory submissions the FDA recommends the following conversion factors: Mouse = 3, Hamster = 4.1, Rat = 6, Guinea Pig = 7.7. (based on Cancer Chemother Repts 50(4):219(1966)) Multiply the conversion factor by the animal dose in mg/kg to obtain the dose in mg/m2 for human dose equivalent. when both height and weight are known, human body surface area is calculated using Boyd's Formula of Body Surface Area (Boyd E. The growth of the surface area of the human body. University of Minnesota Press. 1935) . Calculations with weight alone (no heigth) are less accurate. All values are estimates and values above 2.25 m2 are not considered accurate.

| Species | Weight, kg | Est. Total Dose, mg | Dose in mg/kg | Dose in mg/m2 | Est. BSA,m2 |
|---|---|---|---|---|---|
| Human | 70.00 | 553.03 | 7.70 | 300.00 | 1.843 |
| Mouse | 0.02 | 1.99 | 99.47 | 300.00 | 0.007 |
| Hamster | 0.03 | 2.60 | 86.80 | 300.00 | 0.009 |
| Rat | 0.15 | 7.62 | 50.82 | 300.00 | 0.025 |
| Guinea Pig | 1.00 | 26.70 | 26.70 | 300.00 | 0.089 |
| Rabbit | 2.00 | 47.62 | 23.81 | 300.00 | 0.159 |
| Cat | 2.50 | 59.13 | 23.65 | 300.00 | 0.197 |
| Monkey | 3.00 | 73.63 | 24.54 | 300.00 | 0.245 |
| Dog | 8.00 | 134.40 | 16.80 | 300.00 | 0.448 |

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**Published**
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(54) Title: A METHOD OF ADMINISTERING LIPOSOMAL ENCAPSULATED TAXANE

(57) Abstract

Liposomal–encapsulated taxane or an antineoplastic derivative thereof or a mixture thereof is provided which is used to effect a therapeutically enhanced method of treating cancer. The liposomal encapsulated paclitaxel allows for administration to a patient, particularly a human patient, in less than one hour without substantial toxicity.

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# A METHOD OF ADMINISTERING LIPOSOMAL ENCAPSULATED TAXANE

## TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of administering a liposomal encapsulated taxane.

## BACKGROUND OF THE INVENTION

The use of taxanes, such as paclitaxel, as anti-tumor agents for patients suffering from diseases such as ovarian and breast cancer, is known. In addition, paclitaxel has been shown to be clinically potent as a synergistic agent when used in conjunction with radiation treatment. Paclitaxel has a unique mechanism of action and a broad spectrum of anticancer activity because paclitaxel shows stabilization of microtubules rather than disassembly of microtubules.

However, paclitaxel has extremely low solubility in water, which makes it difficult to provide a suitable dosage form. Currently, paclitaxel is prepared and administered in a vehicle containing Cremophor EL (a polyethoxylated castor oil) and ethanol in a 50:50 (vol/vol) ratio. This solution is diluted 1:10 in saline before being administered to humans. The stability of paclitaxel once diluted in saline solution is quite low. The drug degrades within 24 hours and, thus, handling of dosage for the patients becomes very difficult. Since, the drug precipitates from dilution, an on-line filter is utilized for the infusion of the drug to the patients.

In clinical trials, a consistent problem of anaphylactoid reaction, dyspnea, hypertension, and flushing have been encountered. The dose-limiting toxicity is myelosuppression which necessitates patient hospitalization when the drug is used.

Attempts to prevent paclitaxel cardiotoxicity and anaphylactoid reaction have included reliance on pretreatment of patients with antihistamine and corticosteroids, and by prolonging the infusion time from

six to twenty four hours. U.S. patent number 5,621,001 (Canetta et al.) discloses a prolonged infusion time in a method for reducing peripheral neurotoxicity symptoms while maintaining an anti-tumor effect in patients suffering from ovarian cancer and undergoing paclitaxel therapy. This method involves administering about 135 $mg/m^2$ of paclitaxel over a period of about 24 hours. The administration of paclitaxel is repeated at least once, about 21 days after the preceding administration.

U.S. patent number 5,665,761 (Canetta et al.) discloses a pretreatment stage before administration of paclitaxel. The '761 patent provides for paclitaxel infusions over a duration of less than six hours, preferably about three hours, utilizing dosages of between about 135 $mg/m^2$ and about 275 $mg/m^2$, preferably between about 135 $mg/m^2$ and about 175 $mg/m^2$, after patients had been pretreated to alleviate or minimize hypersensitivity responses. For example, the patients are pre-medicated with steroids, antihistamines, and $H_2$-antagonists sufficient to at least prevent an anaphylactoid shock capable of causing acute hypersensitivity reactions and patient death. U.S. patent number 5,670,537 (Canetta et al.) also discloses this method of administration for a patient suffering from a paclitaxel-sensitive tumor, such as an ovarian tumor.

U.S. Patent No. 5,641,803, discloses the administration of paclitaxel to a patient, wherein about 135-175 $mg/m^2$ of paclitaxel is administered over a period of about three hours. Such a period purportedly was used to overcome, in part, some of the aforementioned problems associated with short infusion times, such as one hour, which had been employed with the conventional paclitaxel formulations containing polyethoxylated castor oil.

In yet another attempt to address the toxicity concerns of the conventional paclitaxel formulation, U.S. Patent No. 5,696,153 suggests the use of an

administration regimen wherein 45 to 120 mg/m$^2$ of paclitaxel is administered over a period of 60 to 180 minutes, a plurality of times during a 21 day period, with each infusion being separated by an interval of between 4 to 5 days.

However, even with these manipulations of prolonged infusion time and pretreatment of patients with antihistamines and corticosteroids, the patients suffer from serious toxicities which are often fatal. Different agent delivery systems are being utilized to enhance tumor cell-fighting effects of the drug and/or reduce systemic toxicity. Liposomes are one of many carriers that have been developed to help anti-tumor agents become more efficient and less toxic. A "liposome" is a closed structure composed of lipid bi-layers surrounding an internal aqueous space.

U.S. patent number 5,648,090 (Rahman et al.)and U.S. Patent No. 5,424,073 (Rahman et al.) provide a liposomal encapsulated paclitaxel for a method for treating cancer in mammals using such a liposomal-encapsulated paclitaxel, or antineoplastic derivative thereof. The '090 and '073 patents disclose a method of modulating multidrug resistance in cancer cells in a mammalian host by administering to the host a pharmaceutical composition of a therapeutically effective number of liposomes which include a liposome-forming material, cardiolipin, and an agent such as paclitaxel, or an antineoplastic derivative of paclitaxel, or a mixture thereof; and a pharmaceutically acceptable excipient.

Up until the present invention the fastest administration time tolerated by most patients was optimally a three hour time period. Consequently, there is a need for methods for rapidly administering high concentrations of taxane in human cancer patients without inducing a toxic reaction. Such methods would improve the efficacy of taxane therapy and alleviate the discomfort and toxicity associated with previously known

taxane administration methods. The present invention provides such a method.

## SUMMARY OF THE INVENTION

The present invention provides a method of administering relatively high concentrations of taxane to human patients over a short period of time. For example, taxane can be administered to humans in less than an hour in an amount from about 75 to 300 mg/m$^2$. Unique liposomal formulations of taxane or its antineoplastic derivatives facilitate such treatments. The method does not require premedication, as with anti-hypersensitivity agents, and is not accompanied by substantial toxic reactions in human patients. As a result, the present invention provides an improved method for treating cancer with taxane.

These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

The invention may best be understood with reference to the following detailed description of the preferred embodiments.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a method of administering a taxane to a patient, especially a human patient, in need of treatment with a taxane. In part, the present invention provides a delivery system for a taxane to a host which is characterized by the avoidance of solubility problems of a taxane; the improved taxane stability; the avoidance of anaphylactoid reactions and cardiotoxicity; the ability to administer a taxane as a bolus or short infusion, rather than an extended infusion of free taxane; the increased therapeutic efficacy of taxane; and the modulation of multidrug resistance in cancer cells.

The taxane is delivered in the form of a liposomal encapsulated taxane or antineoplastic derivative thereof. Any suitable taxane or derivative can be used in the present method. Suitable taxanes when used in accordance with the disclosed methods provide the aforementioned benefits. Preferably, the taxane is paclitaxel. A suitable derivative of paclitaxel is taxasm. Other suitable taxanes are 7-epipaclitaxel, t-acetyl paclitaxel, 10-desacetyl-paclitaxel, 10-desacetyl-7-epipaclitaxel, 7-xylosylpaclitaxel, 10-desacetyl-7-glutarylpaclitaxel, 7-N,N-dimethylglycylpaclitaxel, 7-L-alanylpaclitaxel, taxotere, and mixtures thereof.

The pharmaceutical composition may also include a suitable cardiolipin. Suitable cardiolipin may be from either a natural or synthetic source. The taxane, such as paclitaxel, is encapsulated in liposomes using the cardiolipin. In addition to cardiolipin, the taxane may be encapsulated in liposomes with phosphatidylcholine and cholesterol. Such lipid compositions provide over 90% encapsulation of the drug in liposomes.

The liposomal encapsulated taxane can be prepared by any suitable process. For example, the taxane or a derivative thereof can be dissolved in a suitable solvent. Generally, suitable solvents are non-polar or slightly polar and can be evaporated without leaving toxic residue behind. Suitable solvents include such diverse solvents as ethanol, methanol, chloroform, butanol or acetone. Cardiolipin can also be dissolved in a suitable solvent as described for taxane and the taxane and the cardiolipin solutions can be mixed. The remaining lipophilic material can be dissolved in a suitable solvent, which may be the same as or different from the taxane containing solvent. The solvent will have low polarity such as chloroform, butanol or a non-polar solvent, such as n-hexane. The solvent mixture containing the taxane and cardiolipin can be mixed with

the solution containing the remaining lipophilic components.

The solvent is removed, from the mixture by a suitable method such as by lyophilization to afford a dry lipid film that contains the drug. The mixture is stored in this form, optionally under an inert gas atmosphere, such as an $N_2$ atmosphere. The dry lipid film can be stored at low temperatures, such as -20° C for extended periods of time until liposomes are hydrated and prior to use.

Liposomes can be formed by adding any suitable solution to the lipid film. Typically, suitable solutions are polar solutions, preferably, aqueous saline solutions. Once the solution is added, liposomes can be formed by mixing, for example, as by vortexing. Where smaller vesicles, such as unilamellar vesicles, are desirable the solution can be sonicated. In certain methods, suitable preparations can be mixtures of multilamellar vesicles and unilamellar vesicles.

The liposome is a closed structure composed of lipid bilayers surrounding an internal aqueous space. Generally, the liposomes may be neutral, negative or positively charged liposomes. For example, positively charged liposomes can be formed from a solution containing phosphatidyl choline, cholesterol, and stearyl amine. Negative liposomes can be formed, for example, from solutions containing phosphatidyl choline, cholesterol, and phosphatidyl serine or more preferably, cardiolipin. Other additives can also be included in the liposomes to modify the properties of the resulting preparations. For example, preferred preparations also include α-tocopherol.

Storage conditions can vary. Preferably, mixtures of lipophilic components are stored as dry lipid films at about -20° C. Once hydrated, liposome suspensions of the pharmaceutical composition can be stored and are stable in buffered, neutral pH saline solutions for periods of

hours to months, depending upon the temperature, paclitaxel content, and phospholipid constituents.

The liposomal drug delivery system which features a high drug to carrier ratio can alter drug pharmacokinetics, maintaining the plasma concentration of the drug at an increased level over a longer period of time. The biodegradability and the low inherent toxicity and immunogenicity of liposomal preparations reduces toxicity with respect to free-floating taxanes in the plasma.

The present liposomal formulations provide a drug-delivery system which allows infusion of high concentrations of taxane in a relatively stable form and which provides sustained therapeutic benefits at target sites, while maintaining low concentrations of insoluble free taxane and minimal adverse toxic effects than were previously known. For example, infusion of encapsulated paclitaxel provides higher peak plasma concentrations, longer presence of the drug in the body, and higher AUC ("area under the curve" measurement of plasma concentration over time) than the conventional paclitaxel.

The present pharmaceutical composition can be administered in amounts of at least 50 to 300 mg of active compound/$m^2$ of mammalian host surface area, within a period of less than about three hours, preferably in less than about one hour, and most preferably 45 minutes without causing a substantial toxic reaction. For example, in a 70 kg human, about 0.5 to 5.0 mg active compound per kg of body weight can be safely administered in about 45 minutes. Preferably, about 1.0-3.0 mg of active compound per kg of body weight is administered. Alternatively, preferable amounts include 75, 135, 175, 250, and 300 mg/$m^2$.

Liposomal encapsulated taxane has a substantial beneficial effect in overcoming multidrug resistance in cancer cells which are subjected to chemotherapy. By

using the liposomal composition of the present invention, it is possible to reduce the tendency of cancer cells subjected to chemotherapy to develop resistance to the chemotherapeutic agents used for chemotherapy such as anthracycline glycosides. This method includes administering to a host a pharmaceutical composition of a liposomal encapsulated taxane of the present invention in accordance with the administration protocol.

Taxanes and the anti-neoplastic derivatives thereof may be used to treat any form of mammalian cancer. Such compounds are thought to function by promoting the assembly of microtubules or prohibiting the tubulin disassembly process. Taxane and the anti-neoplastic derivatives thereof are of particular advantageous use in the treatment of mammalian lymphoma, ovarian, breast, lung and colon cancer, and particularly those conditions in humans.

The present liposome compositions can be administered intravenously, intraperitoneally, to an isolated portion of a mammalian body particularly a human body, such as an arm or leg, or in the case of a human, a hand, or can be injected directly into a tumor.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

**EXAMPLE 1**

Paclitaxel can be encapsulated in liposomes of cardiolipin, phosphatidylcholine, cholesterol and ∝-tocopherol. The composition described in this example, provides for over 90% encapsulation of the drug in liposomes. The paclitaxel in liposomal formulation is stable for days at room temperature and at -20° C for at least 5 months. No degradation or precipitation of paclitaxel is observed at any storage temperature and the preparation appears to be ideally suited for systemic administration in accordance with the present invention.

The proportion of lipids per mg of paclitaxel is:

1.8 mg cardiolipin
9.0 mg phophatidylcholine
3.0 mg cholesterol
0.1 mg ∝-tocopheryl

The liposome encapsulated paclitaxel can be manufactured using the following procedure.

8.89 kilograms of t-butyl alcohol are added to a 12.0 liter flask and heated to 40-45° C. The following additions are made sequentially with mixing until dissolution and heating at 40-45° C: 3.412 grams of D-α-tocopheryl acid succinate, 205 grams of egg phosphatidylcholine, 22.78 grams of paclitaxel, 41.00 grams of tetramyristoyl cardiolipin, 68.33 grams of cholesterol.

The resulting solution is filtered through a 0.22 micron filter. The resulting filtrate is filled into sterile vials, each containing about 10.1 grams of filtrate. The vials are stoppered and subjected to lyophilization. They can be stored at -20° C until use.

Liposomes are prepared from the dry lipid film, as needed, with 25 ml of normal saline solution. The mixture is allowed to hydrate at room temperature for about one hour, after which time the vials are vortexed for about one minute and sonicated for about 10 minutes in a bath type sonicator at maximum frequency. An appropriate amount of the contents of the vial can be transferred to an infusion bag and infused into a patient in accordance with the present invention.

**EXAMPLE 2**

The following study demonstrates that a large quantity of taxane can be rapidly administered to humans without inducing a substantial toxic reaction. Both hematological toxicity and nonhematological toxicity were evaluated. In addition, the study was used to determine in human patients the dose-limiting toxicity, the maximum

tolerated dose and the untolerated dose for the liposomal formulation described in Example 1.

Vials containing liposomal paclitaxel were prepared as in Example 1. The preparations were 1 mg/ml paclitaxel in liposomes. The contents of the vials were transferred to infusion bags at the appropriate dosages and administered to patients over about a 45 minute period.

Patients selected for the study had a measurable or evaluable metastatic or locally recurrent malignancy and had no significant hope of cure or palliation by other conventional therapies. In addition, they had no evidence of spinal cord compression or carcinomatous meningitis. Patients had not undergone chemotherapy or radiotherapy within the four weeks prior to treatment. Those patients that had undergone prior chemotherapy or radiotherapy exhibited complete hematologic recovery prior to treatment in this study. All patients had an ECOG (Eastern Cooperative Oncology Group) performance status of 0 or 1 and had a life expectancy of at least 3 months. Patients in the study were all over the age of 18, were free of infection and had recovered from the effects of any major surgery which must have occurred more than three weeks prior to entering the study. Within the immediate two weeks prior to the instant tests all patients had a white blood cell count of over $3000/mm^3$, a platelet count of over $100,000/mm^3$, serum creatinine of less than 1.8 mg/dl or creatinine clearance of more than 60/cc/min and serum bilirubin of less than 1.5 mg/dl.

Treatments were administered intravenously over about a 45 minute period. At least three patients were treated at each dosage level. Dosages were about 90 $mg/m^2$, 135 $mg/m^2$, 175 $mg/m^2$, 250 $mg/m^2$, and 300 $mg/m^2$ allowing for normal laboratory and therapeutic dose variation. The formulation was given as a single agent without pretreatment with steroids, antihistamines or

other therapeutic agents such as anaphylaxis inhibitors. Where the treating physician considered it appropriate, treatments were repeated every 21 days. Each patient was subjected to a single treatment regimen.

Hematologic toxicity was evaluated in test patients by taking blood specimens of 5 mls from each patient. Samples were taken just prior to drug infusion, at the end of the infusion (time=0), then at 2, 4, 6, 10, 20, 30, 60, 240 minutes and 24 hours after infusion. The samples were collected in heparinized tubes which were gently inverted after filling to ensure mixing of the heparinized blood. The vials were kept cool until the plasma was isolated from each sample. As soon as practical, the samples were centrifuged at 2000 rpm, for 15 minutes to collect the plasma layer. Approximately 1 or 2 ml of the plasma was transferred to a cryotube which was capped and immediately frozen at -20° C in an upright position until hematological toxicity analysis. Nonhematological toxicity and drug efficacy were also evaluated. The results of this study are shown in Table I below.

Common toxicity grades established by the National Cancer Institute were employed to determine drug toxicity. Dose-limiting toxicity is defined as any grade 3 or higher non-hematologic toxicity for 7 or more days occurring during cycle 1 of chemotherapy. An untolerable dose is defined as the dose level at which at least 1/3 to 2/3 of the patients have dose-limiting toxicity. The maximum tolerated dose level is defined as the dose level at which 0/6 or 1/6 patients experience dose-limiting toxicity and at least 2/3 or 4/6 patients treated at the next higher dose level experience dose-limiting toxicity.

This study demonstrated that a large quantity of taxane could be administered to a human without inducing a substantial hematological or nonhematological toxic reaction. Nonhematological toxicity was generally minor but became more pronounced at the highest dosage level.

Similarly, hematological toxicity was mild but became more pronounced at the highest dosage. At least 300 mg/m$^2$ of taxane could be administered to a human patient in a 45 minute period without inducing substantial hematological toxicity or anaphylaxis. The dose limiting toxicity was about 300 mg/m$^2$ when drug was administered in a 45 minute period. The untolerable and maximum tolerable doses were not determinable from this study but were at least 300 mg/m$^2$. With one exception, the cancer had not progressed or was improved in each of the patients studied.

TABLE I

| Patient Number | Treatment Cycles | Dose ($mg/m^2$) | Heme Toxicity[1] | Nonhematological Toxicity | Best Response | Off study due to |
|---|---|---|---|---|---|---|
| 001 | 2 | 90 | None | HSR[2] | | P.D.[3] |
| 002 | 11+ | 90 | Mild | | Stable | |
| 003 | 6 | 90 | Mild | (Seizure) | Stable | P.D. |
| 004 | 2 | 135 | | HSR | | P.D. |
| 005 | 6 | 135 | Mild | Muscular & hepatic | Stable | elective |
| 006 | 8+ | 135 | Mild | (HA, fever, pharyngitis, wheezing) | Progressed | |
| 007 | 3 | 175 | Mild | (diarrhea) | | P.D. |
| 008 | 2 | 175 | Mild | Mild hepatic | | P.D. |
| 009 | 1 | 175 | Mild | Recurrent HSR; Nausea/fatigue; Mild hepatic | | HSR |
| 010 | 2 | 250 | Mod | (hemoptysis) | | P.D. |
| 011 | 4+ | 250 | Mild | Mild hepatic (HA, diarrhea, chills & sweats) Esophagitis grade3 after cycle 3 | Stable | |
| 012 | 3 | 250 | Mild | Mild hepatic | | P.D. |
| 013 | 2+ | 250 | Mild | Mild GI, HSR | | |
| 014 | 2+ | 300 | Mod | Hepatic, Esophagitis grade 3 | Improved | |
| 015 | 1+ | 300 | Severe | Mild HSR, Hepatic | | |
| 016 | 1+ | 300 | Severe | Esophagitis grade 3 | | |

[1] neutropenia, anemia, thrombopenia
[2] hypersensitivity reaction: flushing, back pain, pruritis
[3] physician or patient discretion

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WE CLAIM:

1. A method of administering taxane to a patient in need of treatment with taxane comprising administering a pharmaceutical composition over a period of less than an hour in an amount from about 75 to 300 mg/m$^2$ wherein said pharmaceutical composition is liposomal encapsulated taxane or an antineoplastic derivative thereof.

2. The method of claim 1 wherein said taxane is selected from the group consisting of paclitaxel, 7-epipaclitaxel, t-acetyl paclitaxel, 10-desacetyl-paclitaxel, 10-desacetyl-7-epipaclitaxel, 7-xylosylpaclitaxel, 10-desacetyl-7-glutarylpaclitaxel, 7-N,N-dimethylglycylpaclitaxel, 7-L-alanylpaclitaxel, taxotere, and mixtures thereof.

3. The method of claim 1 wherein said pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

4. The method of claim 1 wherein said pharmaceutical composition further comprises cardiolipin.

5. The method of claim 4 wherein said cardiolipin is selected from the group consisting of natural cardiolipin and synthetic cardiolipin.

6. The method of claim 1 wherein said amount of said taxane is about 75 mg/m$^2$.

7. The method of claim 1 wherein said amount of said taxane is about 135 mg/m$^2$.

8. The method of claim 1 wherein said amount of said taxane is about 175 mg/m$^2$.

9. The method of claim 1 wherein said amount of said taxane is about 250 mg/m$^2$.

10. The method of claim 1 wherein said amount of said taxane is about 300 mg/m$^2$.

11. The method of claim 1 wherein said patient is suffering from a ovarian cancer, breast cancer, lung cancer or other neoplasm.

12. The method of claim 1 wherein said liposomal encapsulated taxane is administered by intravenous infusion.

13. The method of claim 12 wherein said liposomal encapsulated taxane is administered over a period of 45 minutes.

14. The method of claim 12 wherein said administration of said liposomal encapsulated taxane is repeated at least once every 21 days.

15. The method of claim 1 wherein said administration of said liposome encapsulated taxane administered intraperitoneally to patients suffering from cancer.

16. The method of claim 15 wherein said administration of said liposome encapsulated taxane is administered intraperitoneally to patients suffering from colon cancer.

17. A method of treating a human with taxane comprising administering rapidly a large quantity of liposomal taxane to a human without inducing a substantial toxic reaction.

18. The method of claim 17 in which the liposomal taxane is administered intravenously.

19. The method of claim 17 in which the liposomal taxane is administered as a single agent without pretreatment by steroids, antihistamines or other therapeutic agents.

20. The method of claim 17 in which substantial nonhematological toxicity is not induced.

21. The method of claim 17 in which substantial anaphylaxis is not induced.

22. The method of claim 17 in which the large quantity of liposomal taxane ranges from about 75 to 300 $mg/m^2$.

23. The method of claim 17 in which the large quantity of liposomal taxane ranges from about 90 to 300 $mg/m^2$.

24. The method of claim 17 in which the large quantity of liposomal taxane ranges from about 135 to 300 $mg/m^2$.

25. The method of claim 17 in which the large quantity of liposomal taxane ranges from about 175 to 300 $mg/m^2$.

26. The method of claim 17 in which the large quantity of liposomal taxane ranges from about 175 to 250 $mg/m^2$.

27. The method of claim 17 in which the large quantity of liposomal taxane is about 250 $mg/m^2$.

28. The method of claim 17 in which the liposomal taxane is rapidly administered in less than 3 hours.

29. The method of claim 17 in which the liposomal taxane is rapidly administered in less than 1 hour.

30. The method of claim 17 in which the liposomal taxane is rapidly administered in about 45 minutes.

31. The method of claim 17 further comprising repeating the step of administering rapidly a large quantity of liposomal taxane to a human without inducing substantial hematological or nonhematological toxicity.

32. The method of claim 31 wherein the repeating step occurs in 21 days.

33. A method of treating a human with taxane comprising intravenously administering to a human in about 45 minutes about 175 to 300 $mg/m^2$ of a liposomal taxane formulation that has a dose limiting toxicity of

at least about 300 mg/m$^2$ when administered in about 45 minutes.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/14986

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/127
US CL :424/450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS:
Search terms: taxane, taxol, ?paclitaxel, liposome?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | US 5,424,073 A (RAHMAN et al) 13 June 1995, abstract, Examples and column 8, lines 28-52. | 1-9, 11-12 & 15-26<br>---<br>10, 13-14 & 27-33 |
| Y | US 5,756,537 A (GILL) 26 May 1998, abstract, Figures, column 2, lines 23 through column 3, line 2, column 6, lines 45-67, examples and claims. | 1-33 |
| Y | US 5,683,715 A (BONI et al) 04 November 1997, abstract, examples and claims. | 1-33 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
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| Date of the actual completion of the international search<br>19 AUGUST 1999 | Date of mailing of the international search report<br>18 OCT 1999 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>GOLLAMUDI S KISHORE<br>Telephone No. (703) 308-1235 |

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US007314637B1

(12) **United States Patent**
**Rahman**

(10) **Patent No.: US 7,314,637 B1**
(45) **Date of Patent: *Jan. 1, 2008**

(54) **METHOD OF ADMINISTERING LIPOSOMAL ENCAPSULATED TAXANE**

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( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(2), (4) Date: **Feb. 25, 2000**

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(52) **U.S. Cl.** ..... **424/450**; 514/449; 514/510

(58) **Field of Classification Search** ..... 424/450; 514/449, 510
See application file for complete search history.

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*Primary Examiner*—Gollamudi S. Ksihore

(57) **ABSTRACT**

Liposomal-encapsulated taxane or an antineoplastic derivative thereof or a mixture thereof is provided which is used to effect a therapeutically enhanced method of treating cancer. The liposomal encapsulated paclitaxel allows for administration to a patient, particularly a human patient, in less than one hour without substantial toxicity.

**42 Claims, No Drawings**

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# METHOD OF ADMINISTERING LIPOSOMAL ENCAPSULATED TAXANE

## TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of administering a liposomal encapsulated taxane.

## BACKGROUND OF THE INVENTION

The use of taxanes, such as paclitaxel, as anti-tumor agents for patients suffering from diseases such as ovarian and breast cancer, is known. In addition, paclitaxel has been shown to be clinically potent as a synergistic agent when used in conjunction with radiation treatment. Paclitaxel has a unique mechanism of action and a broad spectrum of anticancer activity because paclitaxel shows stabilization of microtubules rather than disassembly of microtubules.

However, paclitaxel has extremely low solubility in water, which makes it difficult to provide a suitable dosage form. Currently, paclitaxel is prepared and administered in a vehicle containing Cremophor EL (a polyethoxylated castor oil) and ethanol in a 50:50 (vol/vol) ratio. This solution is diluted 1:10 in saline before being administered to humans. The stability of paclitaxel once diluted in saline solution is quite low. The drug degrades within 24 hours and, thus, handling of dosage for the patients becomes very difficult. Since, the drug precipitates from dilution, an on-line filter is utilized for the infusion of the drug to the patients.

In clinical trials, a consistent problem of anaphylactoid reaction, dyspnea, hypertension, and flushing have been encountered. The dose-limiting toxicity is myelosuppression which necessitates patient hospitalization when the drug is used.

Attempts to prevent paclitaxel cardiotoxicity and anaphylactoid reaction have included reliance on pretreatment of patients with antihistamine and corticosteroids, and by prolonging the infusion time from six to twenty four hours. U.S. Pat. No. 5,621,001 (Canetta et al.) discloses a prolonged infusion time in a method for reducing peripheral neurotoxicity symptoms while maintaining an anti-tumor effect in patients suffering from ovarian cancer and undergoing paclitaxel therapy. This method involves administering about 135 $mg/m^2$ of paclitaxel over a period of about 24 hours. The administration of paclitaxel is repeated at least once, about 21 days after the preceding administration.

U.S. Pat. No. 5,665,761 (Canetta et al.) discloses a pretreatment stage before administration of paclitaxel. The '761 patent provides for paclitaxel infusions over a duration of less than six hours, preferably about three hours, utilizing dosages of between about 135 $mg/m^2$ and about 275 $mg/m^2$, preferably between about 135 $mg/m^2$ and about 175 $mg/m^2$, after patients had been pretreated to alleviate or minimize hypersensitivity responses. For example, the patients are pre-medicated with steroids, antihistamines, and $H_2$-antagonists sufficient to at least prevent an anaphylactoid shock capable of causing acute hypersensitivity reactions and patient death. U.S. Pat. No. 5,670,537 (Canetta et al.) also discloses this method of administration for a patient suffering from a paclitaxel-sensitive tumor, such as an ovarian tumor.

U.S. Pat. No. 5,641,803, discloses the administration of paclitaxel to a patient, wherein about 135-175 $mg/m^2$ of paclitaxel is administered over a period of about three hours. Such a period purportedly was used to overcome, in part, some of the aforementioned problems associated with short infusion times, such as one hour, which had been employed with the conventional paclitaxel formulations containing polyethoxylated castor oil.



In yet another attempt to address the toxicity concerns of the conventional paclitaxel formulation, U.S. Pat. No. 5,696,153 suggests the use of an administration regimen wherein 45 to 120 $mg/m^2$ of paclitaxel is administered over a period of 60 to 180 minutes, a plurality of times during a 21 day period, with each infusion being separated by an interval of between 4 to 5 days.

However, even with these manipulations of prolonged infusion time and pretreatment of patients with antihistamines and corticosteroids, the patients suffer from serious toxicities which are often fatal. Different agent delivery systems are being utilized to enhance tumor cell-fighting effects of the drug and/or reduce systemic toxicity. Liposomes are one of many carriers that have been developed to help anti-tumor agents become more efficient and less toxic. A "liposome" is a closed structure composed of lipid bilayers surrounding an internal aqueous space.

U.S. Pat. No. 5,648,090 (Rahman et al.) and U.S. Pat. No. 5,424,073 (Rahman et al.) provide a liposomal encapsulated paclitaxel for a method for treating cancer in mammals using such a liposomal-encapsulated paclitaxel, or antineoplastic derivative thereof. The '090 and '073 patents disclose a method of modulating multidrug resistance in cancer cells in a mammalian host by administering to the host a pharmaceutical composition of a therapeutically effective number of liposomes which include a liposome-forming material, cardiolipin, and an agent such as paclitaxel, or an antineoplastic derivative of paclitaxel, or a mixture thereof; and a pharmaceutically acceptable excipient.

Up until the present invention the fastest administration time tolerated by most patients was optimally a three hour time period. Consequently, there is a need for methods for rapidly administering high concentrations of taxane in human cancer patients without inducing a toxic reaction. Such methods would improve the efficacy of taxane therapy and alleviate the discomfort and toxicity associated with previously known taxane administration methods. The present invention provides such a method.

## SUMMARY OF THE INVENTION

The present invention provides a method of administering relatively high concentrations of taxane to human patients over a short period of time. For example, taxane can be administered to humans in less than an hour in an amount from about 75 to 300 $mg/m^2$. Unique liposomal formulations of taxane or its antineoplastic derivatives facilitate such treatments. The method does not require premedication, as with anti-hypersensitivity agents, and is not accompanied by substantial toxic reactions in human patients. As a result, the present invention provides an improved method for treating cancer with taxane.

These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

The invention may best be understood with reference to the following detailed description of the preferred embodiments.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a method of administering a taxane to a patient, especially a human patient, in need of

treatment with a taxane. In part, the present invention provides a delivery system for a taxane to a host which is characterized by the avoidance of solubility problems of a taxane; the improved taxane stability; the avoidance of anaphylactoid reactions and cardiotoxicity; the ability to administer a taxane as a bolus or short infusion, rather than an extended infusion of free taxane; the increased therapeutic efficacy of taxane; and the modulation of multidrug resistance in cancer cells.

The taxane is delivered in the form of a liposomal encapsulated taxane or antineoplastic derivative thereof. Any suitable taxane or derivative can be used in the present method. Suitable taxanes when used in accordance with the disclosed methods provide the aforementioned benefits. Preferably, the taxane is paclitaxel. A suitable derivative of paclitaxel is taxasm. Other suitable taxanes are 7-epipaclitaxel, t-acetyl paclitaxel, 10-desacetyl-paclitaxel, 10-desacetyl-7-epipaclitaxel, 7-xylosylpaclitaxel, 10-desacetyl-7-glutarylpaclitaxel, 7-N,N-dimethylglycylpaclitaxel, 7-L-alanylpaclitaxel, taxotere, and mixtures thereof.

The pharmaceutical composition may also include a suitable cardiolipin. Suitable cardiolipin may be from either a natural or synthetic source. The taxane, such as paclitaxel, is encapsulated in liposomes using the cardiolipin. In addition to cardiolipin, the taxane may be encapsulated in liposomes with phosphatidylcholine and cholesterol. Such lipid compositions provide over 90% encapsulation of the drug in liposomes.

The liposomal encapsulated taxane can be prepared by any suitable process. For example, the taxane or a derivative thereof can be dissolved in a suitable solvent. Generally, suitable solvents are non-polar or slightly polar and can be evaporated without leaving toxic residue behind. Suitable solvents include such diverse solvents as ethanol, methanol, chloroform, butanol or acetone. Cardiolipin can also be dissolved in a suitable solvent as described for taxane and the taxane and the cardiolipin solutions can be mixed. The remaining lipophilic material can be dissolved in a suitable solvent, which may be the same as or different from the taxane containing solvent. The solvent will have low polarity such as chloroform, butanol or a non-polar solvent, such as n-hexane. The solvent mixture containing the taxane and cardiolipin can be mixed with the solution containing the remaining lipophilic components.

The solvent is removed, from the mixture by a suitable method such as by lyophilization to afford a dry lipid film that contains the drug. The mixture is stored in this form, optionally under an inert gas atmosphere, such as an $N_2$ atmosphere. The dry lipid film can be stored at low temperatures, such as −20° C. for extended periods of time until liposomes are hydrated and prior to use.

Liposomes can be formed by adding any suitable solution to the lipid film. Typically, suitable solutions are polar solutions, preferably, aqueous saline solutions. Once the solution is added, liposomes can be formed by mixing, for example, as by vortexing. Where smaller vesicles, such as unilamellar vesicles, are desirable the solution can be sonicated. In certain methods, suitable preparations can be mixtures of multilamellar vesicles and unilamellar vesicles.

The liposome is a closed structure composed of lipid bilayers surrounding an internal aqueous space. Generally, the liposomes may be neutral, negative or positively charged liposomes. For example, positively charged liposomes can be formed from a solution containing phosphatidyl choline, cholesterol, and stearyl amine. Negative liposomes can be formed, for example, from solutions containing phosphatidyl choline, cholesterol, and phosphatidyl serine or more preferably, cardiolipin. Other additives can also be included in the liposomes to modify the properties of the resulting preparations. For example, preferred preparations also include α-tocopherol.

Storage conditions can vary. Preferably, mixtures of lipophilic components are stored as dry lipid films at about −20° C. Once hydrated, liposome suspensions of the pharmaceutical composition can be stored and are stable in buffered, neutral pH saline solutions for periods of hours to months, depending upon the temperature, paclitaxel content, and phospholipid constituents.

The liposomal drug delivery system which features a high drug to carrier ratio can alter drug pharmacokinetics, maintaining the plasma concentration of the drug at an increased level over a longer period of time. The biodegradability and the low inherent toxicity and immunogenicity of liposomal preparations reduces toxicity with respect to free-floating taxanes in the plasma.

The present liposomal formulations provide a drug-delivery system which allows infusion of high concentrations of taxane in a relatively stable form and which provides sustained therapeutic benefits at target sites, while maintaining low concentrations of insoluble free taxane and minimal adverse toxic effects than were previously known. For example, infusion of encapsulated paclitaxel provides higher peak plasma concentrations, longer presence of the drug in the body, and higher AUC ("area under the curve" measurement of plasma concentration over time) than the conventional paclitaxel.

The present pharmaceutical composition can be administered in amounts of at least 50 to 300 mg of active compound/$m^2$ of mammalian host surface area, within a period of less than about three hours, preferably in less than about one hour, and most preferably 45 minutes without causing a substantial toxic reaction. For example, in a 70 kg human, about 0.5 to 5.0 mg active compound per kg of body weight can be safely administered in about 45 minutes. Preferably, about 1.0-3.0 mg of active compound per kg of body weight is administered. Alternatively, preferable amounts include 75, 135, 175, 250, and 300 mg/$m^2$.

Liposomal encapsulated taxane has a substantial beneficial effect in overcoming multidrug resistance in cancer cells which are subjected to chemotherapy. By using the liposomal composition of the present invention, it is possible to reduce the tendency of cancer cells subjected to chemotherapy to develop resistance to the chemotherapeutic agents used for chemotherapy such as anthracycline glycosides. This method includes administering to a host a pharmaceutical composition of a liposomal encapsulated taxane of the present invention in accordance with the administration protocol.

Taxanes and the anti-neoplastic derivatives thereof may be used to treat any form of mammalian cancer. Such compounds are thought to function by promoting the assembly of microtubules or prohibiting the tubulin disassembly process. Taxane and the anti-neoplastic derivatives thereof are of particular advantageous use in the treatment of mammalian lymphoma, ovarian, breast, lung and colon cancer, and particularly those conditions in humans.

The present liposome compositions can be administered intravenously, intraperitoneally, to an isolated portion of a mammalian body particularly a human body, such as an arm or leg, or in the case of a human, a hand, or can be injected directly into a tumor.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

## EXAMPLE 1

Paclitaxel can be encapsulated in liposomes of cardiolipin, phosphatidylcholine, cholesterol and ∝-tocopherol. The composition described in this example, provides for over 90% encapsulation of the drug in liposomes. The paclitaxel in liposomal formulation is stable for days at room temperature and at −20° C. for at least 5 months. No degradation or precipitation of paclitaxel is observed at any storage temperature and the preparation appears to be ideally suited for systemic administration in accordance with the present invention.

The proportion of lipids per mg of paclitaxel is:

1.8 mg cardiolipin
9.0 mg phophatidylcholine
3.0 mg cholesterol
0.1 mg ∝-tocopheryl

The liposome encapsulated paclitaxel can be manufactured using the following procedure.

8.89 kilograms of t-butyl alcohol are added to a 12.0 liter flask and heated to 40-45° C. The following additions are made sequentially with mixing until dissolution and heating at 40-45° C.: 3.412 grams of D-α-tocopheryl acid succinate, 205 grams of egg phosphatidylcholine, 22.78 grams of paclitaxel, 41.00 grams of tetramyristoyl cardiolipin, 68.33 grams of cholesterol.

The resulting solution is filtered through a 0.22 micron filter. The resulting filtrate is filled into sterile vials, each containing about 10.1 grams of filtrate. The vials are stoppered and subjected to lyophilization. They can be stored at −20° C. until use.

Liposomes are prepared from the dry lipid film, as needed, with 25 ml of normal saline solution. The mixture is allowed to hydrate at room temperature for about one hour, after which time the vials are vortexed for about one minute and sonicated for about 10 minutes in a bath type sonicator at maximum frequency. An appropriate amount of the contents of the vial can be transferred to an infusion bag and infused into a patient in accordance with the present invention.

## EXAMPLE 2

The following study demonstrates that a large quantity of taxane can be rapidly administered to humans without inducing a substantial toxic reaction. Both hematological toxicity and nonhematological toxicity were evaluated. In addition, the study was used to determine in human patients the dose-limiting toxicity, the maximum tolerated dose and the untolerated dose for the liposomal formulation described in Example 1.

Vials containing liposomal paclitaxel were prepared as in Example 1. The preparations were 1 mg/ml paclitaxel in liposomes. The contents of the vials were transferred to infusion bags at the appropriate dosages and administered to patients over about a 45 minute period.

Patients selected for the study had a measurable or evaluable metastatic or locally recurrent malignancy and had no significant hope of cure or palliation by other conventional therapies. In addition, they had no evidence of spinal cord compression or carcinomatous meningitis. Patients had not undergone chemotherapy or radiotherapy within the four weeks prior to treatment. Those patients that had undergone prior chemotherapy or radiotherapy exhibited complete hematologic recovery prior to treatment in this study. All patients had an ECOG (Eastern Cooperative Oncology Group) performance status of 0 or 1 and had a life expectancy of at least 3 months. Patients in the study were all over the age of 18, were free of infection and had recovered from the effects of any major surgery which must have occurred more than three weeks prior to entering the study. Within the immediate two weeks prior to the instant tests all patients had a white blood cell count of over 3000/$mm^3$, a platelet count of over 100,000/$mm^3$, serum creatinine of less than 1.8 mg/dl or creatinine clearance of more than 60/cc/min and serum bilirubin of less than 1.5 mg/dl.

Treatments were administered intravenously over about a 45 minute period. At least three patients were treated at each dosage level. Dosages were about 90 mg/$m^2$, 135 mg/$m^2$, 175 mg/$m^2$, 250 mg/$m^2$, and 300 mg/$m^2$ allowing for normal laboratory and therapeutic dose variation. The formulation was given as a single agent without pretreatment with steroids, antihistamines or other therapeutic agents such as anaphylaxis inhibitors. Where the treating physician considered it appropriate, treatments were repeated every 21 days. Each patient was subjected to a single treatment regimen.

Hematologic toxicity was evaluated in test patients by taking blood specimens of 5 mls from each patient. Samples were taken just prior to drug infusion, at the end of the infusion (time=0), then at 2, 4, 6, 10, 20, 30, 60, 240 minutes and 24 hours after infusion. The samples were collected in heparinized tubes which were gently inverted after filling to ensure mixing of the heparinized blood. The vials were kept cool until the plasma was isolated from each sample. As soon as practical, the samples were centrifuged at 2000 rpm, for 15 minutes to collect the plasma layer. Approximately 1 or 2 ml of the plasma was transferred to a cryotube which was capped and immediately frozen at −20° C. in an upright position until hematological toxicity analysis. Nonhematological toxicity and drug efficacy were also evaluated. The results of this study are shown in Table I below.

Common toxicity grades established by the National Cancer Institute were employed to determine drug toxicity. Dose-limiting toxicity is defined as any grade 3 or higher non-hematologic toxicity for 7 or more days occurring during cycle 1 of chemotherapy. An untolerable dose is defined as the dose level at which at least 1/3 to 2/3 of the patients have dose-limiting toxicity. The maximum tolerated dose level is defined as the dose level at which 0/6 or 1/6 patients experience dose-limiting toxicity and at least 2/3 or 4/6 patients treated at the next higher dose level experience dose-limiting toxicity.

This study demonstrated that a large quantity of taxane could be administered to a human without inducing a substantial hematological or nonhematological toxic reaction. Nonhematological toxicity was generally minor but became more pronounced at the highest dosage level. Similarly, hematological toxicity was mild but became more pronounced at the highest dosage. At least 300 mg/$m^2$ of taxane could be administered to a human patient in a 45 minute period without inducing substantial hematological toxicity or anaphylaxis. The dose limiting toxicity was about 300 mg/$m^2$ when drug was administered in a 45 minute period. The untolerable and maximum tolerable doses were not determinable from this study but were at least 300 mg/$m^2$. With one exception, the cancer had not progressed or was improved in each of the patients studied.

TABLE I

| Patient Number | Treatment Cycles | Dose ($mg/m^2$) | Heme Toxicity[1] | Nonhematological Toxicity | Best Response | Off study due to |
|---|---|---|---|---|---|---|
| 001 | 2 | 90 | None | HSR[2] | | P.D.[3] |
| 002 | 11+ | 90 | Mild | | Stable | |
| 003 | 6 | 90 | Mild | (Seizure) | Stable | P.D. |
| 004 | 2 | 135 | | HSR | | P.D. |
| 005 | 6 | 135 | Mild | Muscular & hepatic | Stable | elective |
| 006 | 8+ | 135 | Mild | (HA, fever, pharyngitis, wheezing) | Progressed | |
| 007 | 3 | 175 | Mild | (diarrhea) | | P.D. |
| 008 | 2 | 175 | Mild | Mild hepatic | | P.D. |
| 009 | 1 | 175 | Mild | Recurrent HSR; Nausea/fatigue; Mild hepatic | | HSR |
| 010 | 2 | 250 | Mod | (hemoptysis) | | P.D. |
| 011 | 4+ | 250 | Mild | Mild hepatic (HA, diarrhea, chills & sweats) Esophagitis grade 3 after cycle 3 | Stable | |
| 012 | 3 | 250 | Mild | Mild hepatic | | P.D. |
| 013 | 2+ | 250 | Mild | Mild GI, HSR | | |
| 014 | 2+ | 300 | Mod | Hepatic, Esophagitis grade 3 | Improved | |
| 015 | 1+ | 300 | Severe | Mild HSR, Hepatic | | |
| 016 | 1+ | 300 | Severe | Esophagitis grade 3 | | |

[1]neutropenia, anemia, thrombopenia
[2]hypersensitivity reaction: flushing, back pain, pruritis
[3]physician or patient discretion

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

I claim:

1. A method of administering a taxane to a human patient in need of treatment with a taxane comprising administering a pharmaceutical composition to said human patient over a period between about 45 minutes to about one hour in an amount from about 135 $mg/m^2$ to about 300 $mg/m^2$ wherein said pharmaceutical composition is a liposomal encapsulated taxane.

2. The method of claim 1, wherein said taxane is selected from the group consisting of paclitaxel, 7-epipaclitaxel, t-acetyl paclitaxel, 10-desacetyl-paclitaxel, 10-desacetyl-7-epipaclitaxel, 7-xylosylpaclitaxel, 10-desacetyl-7-glutarylpaclitaxel, 7-N,N-dimethylglycylpaclitaxel, 7-L-alanylpaclitaxel, taxotere, and mixtures thereof.

3. The method of claim 1, wherein said pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

4. The method of claim 1, wherein said pharmaceutical composition further comprises cardiolipin.

5. The method of claim 4, wherein said cardiolipin is selected from the group consisting of natural cardiolipin and synthetic cardiolipin.

6. The method of claim 1, wherein said amount of said taxane is about 135 $mg/m^2$.

7. The method of claim 1, wherein said amount of said taxane is about 175 $mg/m^2$.

8. The method of claim 1, wherein said amount of said taxane is about 250 $mg/m^2$.

9. The method of claim 1, wherein said amount of said taxane is about 300 $mg/m^2$.

10. The method of claim 1, wherein said patient is suffering from ovarian cancer, breast cancer, lung cancer, lymphoma or colon cancer.

11. The method of claim 1, wherein said liposomal encapsulated taxane is administered by intravenous infusion.

12. The method of claim 1, wherein said administration of said liposomal encapsulated taxane is repeated at least once every 21 days.

13. The method of claim 1, wherein said administration of said liposome encapsulated taxane is administered intraperitoneally to patients suffering from cancer.

14. The method of claim 13, wherein said administration of said liposome encapsulated taxane is administered intraperitoneally to patients suffering from colon cancer.

15. A method of treating a human having cancer with a taxane comprising administering a large quantity of a liposomal taxane to said human between about 45 minutes to about one hour without inducing a substantial toxic reaction.

16. The method of claim 15 in which the liposomal taxane is administered intravenously.

17. The method of claim 15 in which the liposomal taxane is administered as a single agent without pretreatment by steroids, antihistamines, $H_2$-antagonists or antihypersensitivity agents.

18. The method of claim 15 in which substantial nonhematological toxicity is not induced.

19. The method of claim 15 in which substantial anaphylaxis is not induced.

20. The method of claim 15 in which the large quantity of the liposomal taxane ranges from about 135 $mg/m^2$ to 300 $mg/m^2$.

21. The method of claim 15 in which the large quantity of the liposomal taxane ranges from about 175 mg/m$^2$ to 300 mg/m$^2$.

22. The method of claim 15 in which the large quantity of the liposomal taxane ranges from about 175 mg/m$^2$ to 250 mg/m$^2$.

23. The method of claim 15 in which the large quantity of the liposomal taxane is about 250 mg/m$^2$.

24. The method of claim 15, in which the liposomal taxane is rapidly administered between about 45 minutes and about one hour.

25. The method of claim 15, in which the liposomal taxane is rapidly administered in about 45 minutes.

26. The method of claim 15 further comprising repeating the step of administering a large quantity of a liposomal taxane to a human between about 45 minutes to about one hour without inducing a substantial toxic reaction.

27. The method of claim 26 wherein the repeating step occurs in 21 days.

28. A method of administering a taxane to a human patient in need of treatment with taxane comprising administering a pharmaceutical composition to said human patient in an amount of at least about 300 mg/m$^2$ over a period of between about 45 minutes to about one hour wherein said pharmaceutical composition is a liposomal encapsulated taxane.

29. A method of treating a human with a taxane comprising administering a liposomal taxane ranging from about 75 mg/m$^2$ to about 300 mg/m$^2$ to a human between about 45 minutes to about one hour without inducing a substantial toxic reaction.

30. The method of claim 29 in which the liposomal taxane is administered intravenously.

31. The method of claim 29 in which the liposomal taxane is administered as a single agent without pretreatment by steroids, antihistamines, $H_2$-antagonists or antihypersensitivity agents.

32. The method of claim 29 in which substantial nonhematological toxicity is not induced.

33. The method of claim 29 in which substantial anaphylaxis is not induced.

34. The method of claim 29 in which the large quantity of the liposomal taxane ranges from about 90 to 300 mg/m$^2$.

35. The method of claim 29 in which the large quantity of the liposomal taxane ranges from about 135 to 300 mg/m$^2$.

36. The method of claim 29 in which the large quantity of the liposomal taxane ranges from about 175 to 300 mg/m$^2$.

37. The method of claim 29 in which the large quantity of the liposomal taxane ranges from 175 to 300 mg/m$^2$.

38. The method of claim 29 in which the large quantity of the liposomal taxane is about 250 mg/m$^2$.

39. The method of claim 29, in which the liposomal taxane is rapidly administered in about 45 minutes to about one hour.

40. The method of claim 29, in which the liposomal taxane is rapidly administered in about 45 minutes.

41. The method of claim 29, further comprising repeating the step of administering a liposomal taxane ranging from about 75 mg/m$^2$ to about 300 mg/m$^2$ to a human between about 45 minutes to about one hour without inducing substantial hematological or nonhematological toxicity.

42. The method of claim 41, wherein the repeating step occurs in 21 days.

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